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NUTRITION

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For two reasons, we are particularly glad to be able to publish a contribution from SIR EDWARD MELLANBY. *The first, and more obvious, reason is that this number is concerned with nutritional problems, and Mellanby is well known, not only as the official leader of British medical research, but as one of the builders of nutritional science. An essay on nutritional science in medicine from such a source cannot fail to command special interest and attention. The second reason is that Sir Edward Mellanby has been, since* 1941, *Chairman of the Medical Panel which advises the British Council in that part of its work which is directed to the development of medical relations with other countries. Any progress which this work has made has been to a large extent dependent on Sir Edward's advice, encouragement and accessibility.*

Most readers will know that it was Mellanby who proved rickets to be a disease primarily due to the absence from the diet of a fat-soluble factor (later to be identified as vitamin D). It is perhaps less well known that he was also the first to show (in 1922) *that cereals exerted a rachitogenic influence. In* 1939, *he (with D. C. Harrison) identified phytic acid as one of the factors responsible for this influence. Phytic acid exercises its rachitogenic action by precipitation of dietary calcium as the insoluble calcium phytate. It was an idea of considerable novelty that something in the nature of a simple test-tube reaction, taking place in the alimentary canal on admixture of the reagents concerned, could affect the availability of an important nutrient. This work was extended by investigations on the effect of brown bread on mineral metabolism (see notes on Dr. McCance and Dr. Widdowson below), and has led to the fortification with calcium of the British war-time loaf. Mellanby has recently (Nature,* 1944, **154,** 394) *drawn attention to the complex factors which may affect the amount of phytic acid and its hydrolytic products present in cereal foods in the form in which they are consumed.*

Mellanby has also in recent years been responsible for another piece of research which may well throw light on a number of unsolved clinical problems. This was the discovery that vitamin A influences the growth and shape of bones and, indirectly, the function of the central nervous system and its nerves (including those pertaining to the special senses) as they traverse bony canals. We have already published brief references to this work (BMB **97** *&* **376**), *which was recently summarised in Mellanby's Croonian Lecture to the Royal Society (Proc. roy. Soc. B,* 1944, **132,** 28).

Mellanby's earlier scientific activity has included an investigation into the effects of alcohol on the human organism, made in 1918 *at the request of the British Government, and also work in the fields of cancer and endocrinology. From* 1930 *he was actively associated with the Health Section of the League of Nations and was Chairman of the International Conference for Standardization of Vitamins in* 1931 *and* 1934 *and of the International Technical Commission on Nutrition. Among nutritionists he is regarded as the driving force behind the establishment of international standards of vitamins and of standards for optimal human nutrition.*

In 1933 *he became Secretary of the Medical Research Council, the second to hold that office, and under his administration the Council has initiated or supported an extraordinarily wide field of medical research activities. Since* 1939, *more long-range activities have had to yield precedence to medical problems of the war, and a full account of these will make interesting and impressive reading when it can be published. In particular, the work of the committees, under Mellanby's chairmanship, which have been responsible for the study of numerous physiological and psychological problems of the fighting personnel of the Navy, Army and Royal Air Force, will deserve attention. For an authoritative and thoughtful discussion of problems of the organization and direction of medical research in normal times, the reader should refer to Mellanby's Harveian Oration on 'The State and Medical Research'* (1937).

DR. B. S. PLATT, *the author of our second article, is Director of the Human Nutrition Research Unit recently appointed by the Medical Research Council. Dr. Platt started as a chemist and then turned to the study of medicine. He has had a unique experience of nutritional investigation in the field. From* 1932–38 *he was with the Henry Lester Institute of Medical Research at Shanghai, and while there carried out original investigations on vitamin deficiencies, for which he had a dual qualification as physician and chemist. In* 1938 *he returned to Britain and was appointed to the scientific staff of the Medical*

Research Council as senior member of the Central Organization for Co-ordination of Nutritional Investigations in Colonial Territories. From 1938–40 he was director of a survey in Nyasaland, in the course of which he visited (for the Secretary of State for the Colonies) Uganda, Kenya, Tanganyika, Zanzibar, North and South Rhodesia, and Bechuanaland. In 1940 he became Joint Secretary of the Scientific Committee on Food Policy, which advises the British Government. Between 1940 and 1942 he was responsible for surveys of feeding in industry, in the Royal Air Force, and in the Army, was appointed Secretary of the Medical Research Council committee on the care of shipwrecked personnel, and became a member of several other committees concerned with medical and nutritional problems arising out of the war. In 1943 Platt represented the British Ministry of Health at the United Nations Conference on Food and Agriculture at Hot Springs, U.S.A. In the autumn of 1944 he flew to Newfoundland as one of a survey group, and he has recently left England for a visit to the British West Indies to investigate nutritional conditions (his departure on this mission has made it impossible for him to correct the proofs of his article).

Dr. D. P. Cuthbertson *was in 1943 seconded to the headquarters staff of the Medical Research Council. He is Secretary to the Council's committees on traumatic shock, blood-transfusion research, hæmoglobin surveys, and protein requirements. From 1926–34 he was lecturer in pathological biochemistry at the University of Glasgow, and clinical biochemist to the Glasgow Royal Infirmary. In 1934 he was appointed Grieve Lecturer in physiological chemistry at Glasgow University.*

During the past 14 years his research work has been directed mainly to the study of metabolic responses to injury, factors influencing wound-healing and the strength of bone, and the interrelationships of protein and carbohydrate metabolism. In his article he briefly surveys the difficult and far-reaching subject of protein metabolism.

Dr. S. S. Zilva, *who is a member of the scientific staff of the Medical Research Council working at the Lister Institute, is well known as one of the original workers on vitamin C. He first came to the Lister Institute in 1913 and became a member of the staff of the Medical Research Council six years later. From 1927–31 he was partly seconded to the British Empire Marketing Board for the investigation of nutritional problems in connection with food products from the Empire. He has been a member of the Accessory Food Factors Committee of the Medical Research Council since 1930 and is also a member of others of the Council's sub-committees. For many years he has been engaged in the full-time study of nutritional problems, with special reference to the subject of vitamin C in its chemical, biological, physiological and pathological implications.*

Dr. R. A. McCance *is Reader in Medicine at the University of Cambridge. Originally a research student in physiology, he decided to study medicine at King's College Hospital, London, with which he was associated after graduation. Work with Dr. R. D. Lawrence, the well-known specialist in diabetes, first aroused his interest in the chemical composition of foods. Later, as a result of investigations which lasted 14 years, he published (with E. M. Widdowson) a monograph on the chemical composition of foods, which was issued as No. 235 of the Medical Research Council Special Report Series. McCance's interest in water metabolism was also originally stimulated by work done with Dr. R. D. Lawrence on the secretion of urine in diabetic coma. In 1936 McCance delivered the Goulstonian Lectures at the Royal College of Physicians, taking as his subject his own work upon experimental salt-deficiency in man. The work on salt was followed by a series of investigations on the absorption and excretion of iron, calcium and other minerals (see also note on Dr. Widdowson below). In 1938 McCance was appointed to his present position at Cambridge, but this has involved little interruption of his investigations. Since the war he has devoted particular attention to balance experiments, many of which are performed upon himself and his collaborators, and involve exact measurement of all ingested food and excreta. In 1943 McCance went to Spain and Portugal on behalf of the British Council to lecture and visit medical centres.*

Dr. Winifred F. Young, *who is co-author with Dr. McCance of the article on water metabolism in this Bulletin, studied medicine at Cambridge and was, in 1935, appointed as intern and resident biochemist to the diabetic clinic at King's College Hospital. Since then she has held resident appointments at the South London Hospital for Women and Children, the Infants' Hospital, Vincent Square, London, and the Children's Hospital, Birmingham. She has been the holder of a grant from the Medical Research Council from 1938 to 1943, first (1938–41) for an investigation at Birmingham on the renal function of infants and of dehydration in infants with diarrhœa and vomiting, then (1941–43) for work with Dr. McCance on the study of experimental dehydration in human volunteers at the Department of Medicine, Cambridge.*

Dr. E. M. Widdowson, *who contributes an article on mineral metabolism, started her scientific career as research assistant to the Food Investigation Board of the Department of Scientific and Industrial Research (a State-supported organization parallel in function to the Medical Research Council). From 1933 to 1938 she worked in the Biochemical Department of King's College Hospital and then moved to the Department of Medicine, Cambridge, where she is working as a whole-time member of the Medical Research Council staff. Most of her work has been on the composition of foods and on mineral metabolism in man. In collaboration with McCance, she discovered that iron is never excreted in more than traces, and consequently that the amount absorbed must vary with the needs of the body. The work on mineral metabolism led to the demonstration that brown bread inhibited the absorption of calcium, and provided experimental evidence for the addition of calcium to the war-time flour (which has a relatively high phytic-acid content). Dr. Widdowson also introduced the "individual method" of making dietary surveys, in which each person weighs all that he or she eats for a period of one week.*

Dr. J. D. King *was engaged in full-time research on behalf of the Medical Research Council from 1932-1940. From 1940-41 he held a commission in the Army Dental Corps and conducted an investigation on ulcerative gingivitis. In 1941 he was seconded to the Medical Research Council for further research on parodontal disease, and he has recently been appointed to the Council's whole-time scientific staff at the Nutrition Building of the National Institute for Medical Research. He is the author of a considerable number of original publications on dental and parodontal disease, with special reference to nutritional factors. These publications include one of the Medical Research Council Special Report Series (No. 241) on "Dental Disease in the Island of Lewis".*

Dr. King has co-operated with the Ministry of Health in war-time surveys related to nutrition and oral health.

Dr. J. R. P. O'Brien *is at present demonstrator and lecturer in clinical biochemistry in Oxford University, and biochemist to the Nuffield Scheme for Medical Research. He was a pupil of Professor R. A. Peters, of Oxford, with whom he worked on the isolation and physiological properties of vitamin B_1 and other constituents of the vitamin-B complex. His interests are at present divided between nutritional problems associated with the B-vitamins, and the metabolism of the porphyrin pigments in blood disorders.*

Dr. Magnus Pyke *started his scientific career at McGill University, Montreal, and in 1933 he took a degree in agricultural chemistry. He later carried out research in biochemistry under the direction of Professor, now Sir Jack, Drummond, at University College, London, where he received the degree of Ph.D. of that University and subsequently became a Fellow of the Royal Institute of Chemistry. As assistant to Sir Jack Drummond in the Ministry of Food, he has been concerned particularly with nutritional problems in connection with large-scale feeding. He also acquired experience, both in Canada and in Britain, in applied biochemistry, in the course of work for industrial firms engaged in the manufacture of pharmaceutical and biochemical products.*

SPECIAL CONTRIBUTIONS

NUTRITIONAL SCIENCE IN MEDICINE

Sir EDWARD MELLANBY, K.C.B., F.R.S., M.D., F.R.C.P.
Secretary, Medical Research Council

Nutritional research has now come to occupy a dominant position in the study of problems both of health and of biological science. In regard to disease, it began as a small flame in 1897, when Eijkman correlated his observations on beriberi among Javanese prisoners with his experimental results on polyneuritis gallinarum, finding that these conditions in man and bird were both due to the consumption of polished rice, and preventable by addition of the pericarp of the grain. The flame was fanned by Holst and Frölich in 1907, when they studied scurvy in guinea-pigs and found that it could be prevented and cured by something in fresh vegetables and fruits. As both beriberi and scurvy were rarely seen in western countries, the average medical man was not particularly interested in these clinical discoveries. It was only in 1918, when it was shown by experiments on puppies that rickets, one of the most common diseases of Europe, was due to the absence from the diet of a fat-soluble substance associated with fish and animal fats, that the importance of diet in relation to human health and disease was appreciated.

Recognition of Dietary Deficiency as a Cause of Disease

From 1920 onwards, the flame of dietetic and nutritional research became a fire extending over all countries of the world. In particular, the idea of " deficiency diseases "—that is, diseases due to the absence from the body of specific chemical substances—was generally accepted. It was recognized that, for the maintenance of health, certain vitamins and other accessory factors must be present in the food in sufficient quantities, as the animal body was either incapable of synthesizing them, or its power in this respect was limited.

It is now known that this idea of " deficiency diseases " is not always so simple as when Grijns first formulated it in 1901, and that there are other dietetic and nutritional factors which often determine the development of such illnesses. Thus, the amount of aneurin necessary to prevent beriberi and allied conditions is governed by the amount of carbohydrate or alcohol also consumed ; similarly the amount of vitamin D necessary to produce perfect calcification of bones and teeth is conditioned by the amount of calcium and phosphorus in the food and their relationship to one another. In other words, there are sometimes positive as well as negative factors to be taken into account in such diseases.

It would be unfair, in recording the successes that have followed the establishment of " deficiency disease " as a factor in animal and human life, to forget that the idea of such an ætiology had been advanced so long ago as 1850 by Chatin, following extensive estimations of iodine in foodstuffs, when he related the development of simple goitre to a deficient intake of iodine. This hypothesis was, however, formally suppressed by the French *Académie des Sciences* in 1860. The recognition, as a result of the discoveries of Pasteur, Lister and Koch, that a major cause of disease was the invasion of the body by micro-organisms, was responsible for an outlook which made it difficult, and at the time apparently impossible, to imagine a deficiency or absence of something from the body as being a cause of disease. It was only the discovery by Baumann in 1895 of iodine in the thyroid gland that allowed the resuscitation of Chatin's original hypothesis of the cause of thyroid enlargement, and research from that time has more and more confirmed his views.

If this disregard of dietary deficiency as a factor in disease were the only suppressive effect that could be ascribed to Pasteur's remarkable work, it would probably not be sufficiently important to stress here, but it must also be remembered that it had a similar influence on the attitude to Claude Bernard's hypothesis that many diseases are due to the abnormal working of bodily processes which, when physiological or optimal, result in good health. It will be remembered that Claude Bernard claimed, on this basis, that medical science was essentially experimental physiology. This again was an overstatement of the case, but the success of the *materies morbi* hypothesis, as achieved by Pasteur, Lister and Koch, again delayed, but did not prevent, the ultimate acceptance of Bernard's hypothesis. Indeed the development of research on " deficiency diseases " has tended only to emphasize the truth of the Bernard teaching.

Dangers of a Specialized Outlook

There is surely a lesson to be learnt from these historical facts—namely, that we must avoid allowing the very success of research on the relation of nutrition and disease to cloak and possibly even to suppress, temporarily at least, other pathological hypotheses and theories. The specialist is the backbone of medical, as of all research, but the concentration that leads to discovery is apt to be associated with blindness to other views, and this must be guarded against. Probably the danger is not so great nowadays, when many more men and women are engaged in medical research and when publication of results is so easy and widespread, but it would be well if the danger were more widely recognized. What is more likely to happen now is, not the suppression of new points of view and new lines of scientific investigation, but the too rapid development, and adoption as a fetish by an advertisement-goaded public, of new discoveries in medicine. This undesirable reaction as regards vitamins has not been absent from some countries, and scientific men ought to combat it. Except in infancy and childhood, there are but few instances where good health cannot be maintained by eating properly chosen food without the constant administration of vitamin preparations.

Biological Problems

Surveying now the general field of nutritional science, what do we find? We see that physiologists and biochemists, mainly by means of experiments on the growth of young rats, have detected a large and increasing number of chemical substances which must be present in the diet, often in minute quantities, either for the maintenance of life itself, or for proper growth. The chemical constitution of most of these vitamins is known. The chemists' success has indeed been great. But what about the biological side: can it be said why these substances are essential and/or in what way they work? From this angle the success has not been so outstanding: a biological problem is usually much more difficult than a chemical problem, for the living cell and animal body are not limited in their actions by the known rules and regulations of the chemist's laboratory. Besides, the physiological technique which led to the discovery of many of these substances, namely growth in young animals, is so crude, and the factors involved are so many and complicated, that little knowledge of biological function can be deduced from them.

Thus, there is hardly any knowledge of how the vitamins work in performing their functions. Even when the ultimate action of a vitamin is known, as in the case of vitamin D, whose main function is certainly so to control calcium-phosphorus metabolism as to bring about the calcification of bones and teeth, there is still no knowledge of how it works. Does it simply bring about the absorption from the intestine of sufficient calcium and phosphorus to maintain these elements at an optimum level in the blood, leaving the calcifiable tissues to select their needs from the blood stream, or does it actually assist the calcification process in the bones and teeth? Even when there is sure evidence that vitamin D acts in either or both these ways, there will still remain the problem of how it performs these functions. Does it, for instance, form part of an enzymic complex analogous to the co-carboxylase in which vitamin B_1 is known to participate? In the case of vitamin D we have at least reached the stage of formulating the problem, and the same applies to vitamin K, but with most other vitamins we have not even reached this stage. Clearly this is a great

challenge to all interested in experimental biology—physiologists, biochemists, pathologists, pharmacologists and clinicians. To pathologists the appeal is specially strong, even to those concerned with morbid anatomy, for one outstanding result of modern work on nutrition is that it is gradually making that subject into an experimental science. Few things are more interesting than to see how, one by one, it is becoming possible to reproduce under experimental dietary conditions the long-known classical changes seen in morbid tissues, one of the most interesting in recent times being the fatty infiltration, necrosis and cirrhosis of the liver induced by dietary defects of choline and the amino-acid methionine.

Nutrition and Infection

Nothing seems to be more certain than that nutritional defect is at least part of the ætiological basis of many of the more common illnesses, and yet, in most of these cases, this is only conviction without satisfactory proof. For instance, it will probably be found that a dietetic or nutritional factor is involved in the ætiology of enlarged tonsils and adenoids, peptic ulcer, diabetes mellitus, pernicious anæmia, arteriosclerosis and rheumatic disease. The relation of nutrition to infection remains an unsolved problem, but that there is such a relationship is not to be doubted. Tuberculosis and other infective diseases increase when the nutritional condition of a population is greatly lowered. What is even more certain is that in badly-fed populations infective invasions by micro-organisms are much more deadly than in the well-fed.

In the great field of investigations which lies before us, we must keep a balanced view of the relative importance of the two factors—infection and resistance to infection—as influenced by nutrition. The bacteriologist and epidemiologist may, at times, be inclined to lay all stress on the infective process and the pathogenicity of the micro-organisms; the nutritionist, on the resistance of the body. The relative importance of these factors will no doubt vary greatly in each particular infective process studied, but that both must be regarded as of major importance in many cases is undoubted. We have recently seen how fruitful has been the study of some chemotherapeutic agents, especially the sulphonamides, when considered from the point of view of the chemistry of the bacteria upon which they act, and it is not to be doubted that knowledge of the chemical processes which underlie the toxic action of pathogenic micro-organisms and the resistance of the tissues to them, and of how these are affected by nutrition, will shed great light on infective disease. In some cases it may even be found that passage of an infective agent through a series of people in good nutritional state will lower the pathogenicity of the invading micro-organisms.

In some types of infection it will probably be found that diet, acting during the period of development of the affected tissues and after their formation, is the main controlling factor as, for instance, in dental caries, gingivitis and parodontal disease. In other cases good nutritional conditions may only modify the severity of the disease by increasing the resistance of the body to the infective agent, as for instance in tuberculosis, while in some types of infection the diet and nutritional state may have little or no effect on the incidence and severity of the condition.

To what extent, if any, dietary factors will be found to have a specific anti-infective action remains for future investigation. Some investigators on nutrition have already had experience in the search for this El Dorado, and in consequence regard the problem with more respect. Nothing could have been more promising than the indications of a specific anti-infective action of vitamin A in young rats, which die almost without exception with multiple infective foci when deprived of vitamin A, and recover rapidly if this vitamin or carotene is added to the diet within reasonable time. In endeavouring to extend these facts to bacterial infection in human beings two points were not realized. The first, that rats hold a special place among animals in their susceptibility to infection when deprived of vitamin A, because of their great liability to develop hyperplasia and metaplasia of epithelial surfaces under this condition. The second, that it seldom happens that human beings are deprived of vitamin A to the extent produced experimentally in the rat experiments. Although it is undoubtedly true that a great deficiency of vitamin A in the body lowers man's resistance to bacterial infection, it is improbable that a degree of deficiency of this vitamin, such as is met with in Britain and comparable countries, is an ætiological factor in infective disease of great significance, except possibly in infants. Nor is there any substantial evidence in the case of vitamin A that added resistance to infection results from giving more of it than is physiologically needed. Indeed there is but little evidence that giving amounts of any vitamin larger than those physiologically necessary makes the body functionally more effective. An exception to this is nicotinamide which, in larger doses than are necessary for health, appears to produce an effect on the neuro-muscular mechanism in man which lowers the time needed to carry out strenuous exercises associated with good co-ordination.

Nutritional Science and Food Policy

War conditions in Britain have made it essential for the maintenance of health that modern knowledge of nutrition as revealed by research should play an important part in framing food policy. War-time food policy in this country, as carried out by the Ministry of Food, is described in a separate article below.* It might be of interest to say something from the point of view of the scientist on this question. It can be said at once that during war-time nutritional experts in Britain have had an opportunity to express their views and to give their advice either through the *Medical Research Council* or through the Food Policy Committee of the War Cabinet. Generally speaking, actions by the Ministry of Food have followed the lines of this advice, sometimes on the basis of its nutritional advantages to the nation, and occasionally because the shipping and economic conditions enforced its adoption. As the scientists' advice on food policy in war-time was based on both of these factors, it came to the same thing in the end, but this will not necessarily continue to be so when the time comes to adopt a long-term policy for the days of peace. When that time arrives, policies dependent on scientific and economic factors respectively will often be divergent and, unless the nutritional needs of the people are given precedence over economic claims, much of the progress in health standards of the population may be lost.

The war has emphasized the lesson that in industrialized countries two foodstuffs have pre-eminent claims to national control. The first is milk and the second cereals, especially bread. So far as milk is concerned, the policy of Britain is satisfactory and will probably remain so in peace-time. In this case the Government policy to ensure that all pregnant and nursing mothers and all infants and children receive, if they so wish, a reasonable quantity of milk can only be commended. It is true that in war-time many adolescents and adults have had to go short of milk, especially in the winter months, and it is also true that too high a proportion of the milk is neither clean nor bacteriologically safe. Determined action is required both to increase the supplies and to improve the quality, and the Government seems inclined to press on with both of these policies.

The Bread Question

In the case of cereals and cereal products, the situation is not so satisfactory. Bread made from wheat has in the past formed, and undoubtedly will in the future, a substantial part of the British diet. The bread eaten before the war was made of a low-extraction flour (70 to 73% of the berry), and consisted entirely of the endosperm, while the bran, aleurone layer, scutellum and embryo were extracted by the millers and sold for cattle, pig, and poultry food. Thus, most of the vitamin and mineral contents of the wheat berry which are known to be essential for health went to animals, while the residue formed a substantial part of human dietary. Nutritional scientists are agreed that this policy is wrong, but unfortunately there are two schools of thought as to the best method of dealing with this important national problem. One school, represented by British scientists, thinks that bread for human consumption should be made of flour containing as much of the wheat berry as is physiologically absorbable, i.e. the whole grain except for the outer coarse bran. The second school, represented by some North-American scientists, thinks that flour should be of the old low-extraction, white type, with the addition of those vitamins prepared by synthetic or other methods which are known to be present in the original berry. This second method is based on the assumption that the main vitamin contents of cereals are known, and that their physiological importance

* [*see BMB* 492]

to the body is understood. These assumptions many physiologists will find difficult to accept. It is probable that the policy of the British Ministry of Food in war-time as regards bread production, namely of producing 85%-extraction flour with a minimum of fibre and with high vitamin-B_1 and iron content, has depended more upon shipping difficulties than upon nutritional grounds. Consequently, when the shipping situation is eased, there will be a powerful move to return to the meretricious attractions of the old white bread for human consumption, thus releasing to the farmer, poultry-keeper and manufacturer of proprietary food-products more of the valuable offal for their own purposes. Possibly as a gesture to those with knowledge of nutrition there will be added to the bread aneurin, nicotinamide, riboflavin and iron, as is now being done in North America.

This solution of the bread problem, which consists essentially of depriving flour of most of the chemical entities in wheat which are necessary to life and then restoring a few of these substances, seems wrong in principle. In fact, the method is also wrong, because the low-extraction white flour will undoubtedly be more and more eaten in tropical and other countries in which the addition of the vitamins will not take place. There will be a repetition of the polished rice story, only in this case it will be soulless bread.

Need for International Agreement

It is obvious that scientists of different countries cannot afford to differ on this important question of the best utilization of available foodstuffs. In the case of cereals, and particularly bread, it is very desirable that after the war they should come together and see whether they cannot reach agreement on the optimum composition, both from a nutritional and from an æsthetic point of view, of those cereal preparations which form such a large proportion of the normal diet. The path to such an agreement in policy has already been blazed by the Health Section of the League of Nations. This body achieved the outstanding feat, both of setting up international standardization of vitamins, and also of determining standards of nutrition in terms of common foodstuffs. Probably after the war either this body or its successor will be able to bring about further international co-operation in matters of diet and nutrition and, among other things, agree on a common policy as regards the optimal composition of bread and other cereal products. It may be that new milling techniques will have to be introduced to secure the production in peace-time of bread which fully accords with the principles advocated by British scientists: namely, bread made from a flour which contains all the nutritionally important contents of the wheat berry and is at the same time æsthetically excellent.

All that is demanded is that the factor controlling the constitution of bread should be primarily the health of the consumer, and that milling and other interests should be secondary to this condition. Indeed, no nutritional policy adopted by governments can be wrong if it places the health and the needs of the community as its first and guiding principle.

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ASPECTS OF NUTRITIONAL RESEARCH

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The science of nutrition has grown rapidly in recent years; there has been an almost equally rapid application of nutritional knowledge in public health and social and economic problems the world over. In this essay, I want to illustrate from my own work, and from work with which I have been intimately associated, the nature of the most important phases of the progress made in nutritional science and in its practical application.

Types and Grades of Nutritional Deficiency

The advances made in nutritional science in the present century have mainly originated in the study of dietary deficiency disease. It is now well recognized that such diseases as rickets, beriberi, scurvy and pellagra are caused by lack of specific chemical substances or nutrients in the diet. In general, these are primary dietary diseases; symptoms and physical signs similar to those found in these diseases may, however, develop secondarily to conditions in the body which give rise to impaired absorption and utilization, or abnormally increased requirements, of the specific nutrient.

The grade of a deficiency disease, whether primary or secondary, depends on the *degree* of the insufficiency of the food factor and its *duration*; the degree may be mild, moderate or severe, the duration acute, subacute or chronic.

Observations made in a combined biochemical, clinical and field investigation of many hundreds of cases of vitamin-B_1 deficiency in China from 1933 to 1937 (Henry Lester Institute, 1935, 1936a, 1937–38a; Platt & Gin, 1934; Platt & Lu, 1935, 1936) will assist, perhaps better than those on any other deficiency disease, in elucidating the various ways in which a primary dietary deficiency may manifest itself. In acute severe vitamin-B_1 deficiency the patient was obviously seriously ill, was either greatly distressed, or sometimes collapsed, and, before the highly concentrated or pure vitamin B_1 was available, invariably died. Pyruvic acid was isolated from the blood of patients with this acute, severe vitamin-B_1 deficiency. It was found that this substance accumulated in the blood up to a level of 5 to 6 mg. per 100 ml. A single dose of 5 mg. of pure vitamin B_1 was usually sufficient to reduce this to the normal level of about 0·5 mg. per 100 ml. in 12–15 hours, with complete recovery of the patient. In striking contrast to this type is the chronic so-called "dry" beriberi. There is no accumulation of pyruvic acid in the blood of this group of patients, and treatment for long periods appeared to have little or no effect.

By far the largest group of beriberi cases encountered in Shanghai were of subacute, mild or moderate deficiency. There was usually a small accumulation of pyruvic acid in the blood; the signs and symptoms had some of the features of the acute, the severe and the chronic types, but they responded to treatment with vitamin B_1 more slowly than the former but much more markedly than the latter grade.

Observations (Henry Lester Institute, 1936b) on the effect of the treatment of scurvy with pure vitamin C, and of gross follicular keratosis with vitamin-A concentrates, though less extensive than those on beriberi, were sufficient to justify the view that these diseases could exist as primary dietary defects in substantially "pure" forms. At the time this work was done, the components of the vitamin-B_2 complex had not been separated, but pellagrins were observed to recover rapidly on treatment with certain liver concentrates now known to be rich in nicotinic acid.

Factors Contributing to the Clinical Expression of Nutritional Deficiency

Added to the effects of degree and duration on the manifestations of vitamin-B_1 deficiency, were the consequences of various stress factors. These included physical exertion, the amount of rest and sleep obtained, the quantity of carbohydrate foods in the diet, the temperature and humidity of the climate, the co-existence of infections (particularly those associated with fever) and the concurrence with the deficiency state of hyperthyroidism. Thus, the onset of the majority of beriberi cases coincided with the onset of the hot humid season of the year. Patients with the acute severe disease were usually admitted to hospital in the early hours of the morning after a sleepless night due to poor sleeping quarters and bad hygienic conditions, including infestation with bed bugs. Characteristically, patients of this group were young hard-working males, accustomed to eating large meals consisting mainly of highly milled, well washed rice.

The manifestations of deficiency observed in pregnancy are of special interest (Platt, 1937), in that they illustrate how stress factors and duration can contribute at different stages to the appearance of different signs and symptoms. Towards the end of pregnancy, when there is already strain on the

circulatory system, the signs and symptoms of nutritional deficiency are predominantly cardiovascular, such as swelling of the feet towards the end of the day, with breathlessness on exertion. This evidence usually disappears with parturition, and if dietary insufficiency persists and lactation ensues, a well-defined polyneuritis may develop.

Stress factors also play their part in determining the site of a lesion. Thus, the peripheral neuritis of vitamin-B_1 deficiency may be most marked in the most used parts of the body. A man stripping hides with a knife held in the left hand, the right hand holding the hide, showed changes in the left upper limb only; a tailor's cutter had a right "thumb drop" and a tram driver had changes most marked in the upper arms. Pressure may prove to be a localizing factor, for example, follicular keratosis due to vitamin-A deficiency may be found only on the right forearm in women who do not show such changes elsewhere; these women will be found to rest their forearm on their knee when shelling maize or peeling plantains in a squatting position. Exposure to sunlight is a factor in determining the areas of the skin affected in a pellagrin.

In addition to the combination of dietary deficiency and various stresses, zymotic disease factors may play a part in determining or modifying the clinical picture as, for example, in the association of the fusiform bacillus and spirochætes of Vincent's disease in the gums in nicotinic-acid deficiency in sites where there is local trauma or irritation. The organism (*Oidium albicans*) associated with "thrush" may develop on an excoriated area, and an angular stomatitis thus infected becomes "la perleche".

Early Observations on the Occurrence of Minor Deficiency States

Once the ætiology of the frank cases of deficiency diseases, resulting mainly from insufficiency of single food factors, was recognized and the therapeutic measures became available, the centre of interest in malnutrition shifted to the study of minor manifestations of insufficiency of these and other accessory food factors. In a summary of a lecture I gave in 1936 (Platt, 1936a) it is stated:

"Emphasis is laid on the discovery of the prevalence of the manifestations of minor grades of dietetic deficiency diseases, in the study of which it may be taken as a general principle that the knowledge of the natural history of these various diseases and their uncomplicated and more flagrant forms, provides the basis for identification in their larval stages."

Some of these minor manifestations I described as occurring in associations, particularly with the minor manifestation of vitamin-B_1 deficiency, and in summarizing observations of their incidence I wrote (Henry Lester Institute, 1937–38b):

"In 1933–34 the occurrence of various clinical manifestations in association with beriberi was recognized. These were evidently early stages of deficiency diseases not sufficiently well developed to be called scurvy, pellagra, etc. Observations were made on numerous subjects in factories, workshops, schools and on inhabitants of rural areas using these signs as indices of improper nutrition. The results obtained showed that in industrialized areas, especially amongst the younger workers, there was a high incidence of malnutrition measured by this method. Furthermore there were very many cases of minor grades of deficiency for each frank case of deficiency disease recognized. Of nearly two thousand so-called apprentices in small workshops in Shanghai 70% showed at least one deficiency sign. These boys comprised 40% of the total working force. Owing mainly to the deficiency diseases which had developed, a large turnover was found; 60% worked one year or less and in a sample of 200 workers 15% had been replaced in three months. A high proportion of the deficiency states met with were due to vitamin-B_1 deficiency.

"The knowledge of the appearance of these forms of impaired nutrition served as a basis for a general survey of the population in some refugee camps. These results, from samples of 50–100 persons taken from each of six refugee camps containing some 1,500 refugees at the end of August 1937, showed that, in a total of 400 refugees, 117 had evidences of vitamin-B_1 deficiency, 11 of these being established beriberi. These subjects had not been in the camps for more than ten days and the evidences of deficiency must have been due to deficient diets taken before the war situation developed. It is interesting that the distribution of evidences of vitamin-B_1 deficiency in this sample of population is similar to that found by us in the previous year for young factory boys."

Effects on Nutrition of Food Habits and Methods of Preparation of Food

The study under discussion also included an investigation of the dietaries of the patients, most of whom came from small factories where they worked, ate and slept, few surviving two summers without sickening, dying or being sent back to their homes in the country. Their food seemed at first sight to be similar to their home diets in that they ate mainly rice, occasionally wheat products, vegetables, soya bean preparations and a little meat and fish. But when enquiries were extended into the villages from which the city's labour was recruited, important differences emerged. Small, occasional but precious, additions to the dietary made by the mother in the home were not included in the food provided by the city contractor who, in order to cater at the cheapest possible rate, also bought stale vegetables and inferior grades of rice. The rice had not only been highly milled in the first place but often, owing to the growth of a mould during storage, it had to be re-milled and then vigorously washed to make it fit to eat. This high degree of milling, combined with excessive washing, removed all but a small fraction of the vitamin B_1 in the grain. In the rural areas, on the other hand, the rice was usually freshly prepared from grain stored in "paddy" form, i.e. with the husk on, was home-pounded and therefore not highly milled, and did not need to be thoroughly washed. In an experiment carried through in the summer of 1937 and only just completed at the outbreak of the Sino-Japanese war (Platt, 1939), it was found that, by using rice processed by mechanical means, but following lines similar to the traditional methods, the occurrence of signs and symptoms of beriberi could be entirely prevented.

This experiment illustrated the importance of taking into account food habits and practices. Another valuable illustration is afforded in work done on infant feeding in China (Platt, 1936b; Platt & Gin, 1938). A first reaction to this problem, in the absence of cow's milk, is to find a substitute, as witness the attempts to use soya bean milk with various additions. The Chinese in their wisdom, however, point the way to the most satisfactory solution by making every effort to ensure the supply of mother's milk over a long period and, if this fails, to secure human milk by a system of wet-nursing which does not necessarily deprive the foster-mother's own infant.

Social Factors Revealed by an African Survey

In 1938 I began work as a member of the staff of the *Medical Research Council* along the lines set out in the first report of the Committee on Nutrition in the Colonial Empire. This Committee's work may be traced back to the stimulus given to co-ordination of effort in health and agriculture by the Health Organization of the League of Nations, an account of which will be found in the report of the League of Nations Mixed Committee on Nutrition (1937). Part I of the report of the Committee on Nutrition in the Colonial Empire (1939) which, for the most part, can still be recommended for study and application, brings out the many-sided nature of the problem of nutrition under a wide variety of conditions, and on the recommendation of the Research Sub-Committee a combined survey was carried out in Nyasaland in 1938–40. This comprised four sections: (i) economic and agricultural survey; (ii) dietary survey; (iii) physical and clinical survey; (iv) anthropological and social survey. Owing to the war the results of this investigation have not yet been published, but in a brief account given in an address to the Food and Nutrition Board at Washington in June 1943 I summarized some of the more interesting observations.

One example may be given to illustrate how indirect may be the attack required to improve nutrition. In one village studied it was found that on an average every woman spent over 4½ hours a day on four main tasks: pounding maize; carrying water; collecting and carrying wood; collecting materials for side dishes. All this was additional to any work done in the fields—and it must be realized that most of the agricultural work is done by the women—and to time spent on cooking, seasonal tasks like drying leaves, burning plants for potash, pot-making, grass-cutting for thatching, and mud-plastering of hut walls. Any attempt to improve nutrition must depend on the women who, in view of the time spent on such drudgery as the four tasks mentioned, have neither leisure to listen to instruction nor time to learn new ways.

The first step, then, in improving nutrition under such conditions would be the introduction of, say, a small stone mill in the village, and possibly the training of a miller to prepare meal from the staple grain. At once all the women would have a daily average of over 1½ hours to spare for new work. The cattle which, at the present time, are regarded merely as bank notes, might well be trained as draught animals to carry water. Trees in neighbouring tsetse fly-infested areas might be cut down and burned for charcoal to supply fuel. Opening up stream beds and conserving rainfall for irrigation would provide all the vegetables needed near at hand and save the time now spent on collecting greenstuffs from the bush.

Looked at in another way, the problem studied called for specialization in a community whose members were all Jacks-of-all-trades. Such a development would involve the establishment of secondary industries and the training of craftsmen. It might, therefore, be argued that the key to the solution is education: it might also be argued that this could best be done by developing the rudimentary apprentice system already existing, rather than by more academic methods of instruction.

This brief reference will serve to indicate how far it may be necessary to go in seeking a solution to a problem of nutrition, and how important it may be to make investigations on a broad basis. At the time when this work was done it seemed as if the survey was likely to be the best means of determining how to achieve good nutrition in a territory.

Planning for Nutrition

It is true that surveys of this kind still have their place but, as a result of experience gained during the war, my view is that emphasis should now be placed primarily on overall planning on a territorial basis (Platt, 1943).

In the first World War of 1914–1918, the *Royal Society* appointed a Food (War) Committee which examined various problems of nutrition and agricultural production in order to secure the best possible nutrition under war-time conditions. In the present war, in June 1940, the Lord Privy Seal, as Chairman of the Cabinet Committee on Food Policy, appointed the Scientific Food Policy Committee to "consider and advise upon the problems of national food requirements and of home food production with special regard to the shipping and foreign exchange likely to be available for imports of food and animal feeding stuffs and the labour and other resources likely to be available for home production" (International Labour Office, 1942). The results of the work of this body have not yet been published for security reasons, but it must be obvious that the problems in this war are essentially similar to those dealt with by the corresponding committee in the last one. The value of its work cannot be estimated without knowledge of it, but some of its fruits at the executive stage are in the hands of the Ministries of Food, Health, Agriculture and War Transport, and the report of the United Nations Conference on Food and Agriculture (1943a), quoting Great Britain as an example, stated: "Recent experience has demonstrated how effective a considered food and nutrition policy, based on scientific knowledge and experience, can be in safeguarding the health of a population."

In drawing up a scientific food policy the following steps are involved:

i. Determination of the population's needs for health in terms of the more important nutrients: this includes knowledge of population-distribution in respect of infants, children, pregnant and nursing women and occupational groups, with selection of appropriate nutrient allowances. From these data the value for the per-person needs of the population can be calculated.

ii. Translation of the nutritional requirements into terms of foods having regard to the customary foods of the people and the approximate amounts in which they are eaten.

iii. The estimation of existing food supplies both from home production and from imports. It may also be necessary at this stage to consider potential as well as actual home production, taking into account its cost in terms of land and labour, and alternative sources of imports having regard to length of haul, etc., together with losses occurring in the handling and processing of foods, and the extent to which these might be reduced.

iv. Comparison of the aggregate supply with the estimates of requirements based on nutritional needs, so as to detect and measure the gaps between the two and to determine how they should be filled.

v. Arrangements for ensuring equitable distribution of the supplies of food available. In addition to an appreciation of the nutrition problem in terms of state of health, and to knowledge of food habits and customs and agricultural practices, a full understanding of the methods of distribution will be necessary as a basis for formulating nutritional policy in such a way as to secure the co-operation of the people, thereby strengthening the community socially as well as physically.

Some account of the results of this work on scientific planning of British war-time food policy has been given elsewhere in this *Bulletin*.* The organization behind the formulation of the plans, which were carried out by the Ministries of Food, Health and Agriculture, involved the co-ordination of effort by statesmen at the highest level, with the assistance of scientific and technical advice covering a wide field of interests including medicine, agronomy and economics. In fact, the relationship between the scientific advisory body and the nation's Ministers was on a plan essentially similar to that described later in this essay. Much research work and technical investigation were undertaken in planning the nation's requirements under conditions of restricted food supply; one important example is to be found in the *Medical Research Council* (1940, 1941, 1943a) reports on the nutritive value of National Flour. Other enterprises, such as the production of B vitamins and protein by means of *Torula utilis* (Colonial Food Yeast Ltd., 1944) have not, in fact, been undertaken in Britain during the war, but may well prove to be of value in dietaries in other parts of the world in which supplies of these nutrients are unsatisfactory.

A considerable amount of work was carried out early in the war in instituting collective feeding schemes on a sound basis. Developments along these lines have led to a high standard of catering in various branches of the Armed Forces, and also in the control of feeding in industry. Illustrations of other special problems which have resulted in new knowledge and techniques are the design of compact rations for special troops, and guidance as to the best use of water and food supplies in lifeboats (*Medical Research Council*, 1943b).

Food planning in the United Kingdom, both in broad outline and in much of its detail, has been a war-time measure and owes its success largely to the misfortunes of war; will it be possible to extend this success into the peace? The United Nations Conference on Food and Agriculture (1943b) laid down all the plans necessary. Fundamental principles of food production are incorporated in such recommendations as the following (p. 23):

"That, as a first step in overcoming the general shortage of food, every effort should be made by countries whose agriculture can be expanded in the short-term period, so long as this is required and so far as the conditions of individual countries require or permit, to increase the acreage under crops for direct human consumption and even to hold back the rebuilding of depleted livestock herds—essential though this rebuilding will ultimately be—as well as the production of other crops which compete for acreage with essential foods." In regard to co-ordination, the Conference recognized (p. 24) that "it is essential for the preservation of life to secure, through equitable distribution, the maximum advantage from such supplies as may be made available"; and that the goal of governments and authorities should be the achievement of an economy of abundance.

Plans for the future made on a world scale would, in my opinion, have had a better hope of success had health as well as food and agriculture been taken into account. Recognition of the problems of malnutrition has been and always must be the concern of the medical man. No matter how many steps lie between recognition and the application of the remedy, the final assessment of its efficacy must again be a medical matter. How is this direction and co-ordination to be achieved?

The need for co-operation is summarized as follows in the report of the Committee on Nutrition in the Colonial Empire (1943, p. 168):

"A general and substantial improvement in nutrition in the Colonial Empire must depend upon the steady and concerted

* [*see BMB* 492]

efforts over a period of years of many Government departments and voluntary agents. The primary responsibility necessarily rests with the Medical and Health Departments, but it is through the efforts of the Agricultural Department, working in conjunction with the Veterinary Department, that changes and adjustments in the production of foodstuffs must be made. Much can be done also by the Department of Education in contributing to an improvement in nutritional conditions. The efforts of the more specialized departments will not achieve their full effect unless they receive the support and co-operation of the Administration. Of equal importance is the need for securing co-ordination. Local nutrition committees should be capable of exercising an important influence in this direction, and we hope that they will continue as active bodies, and that full use will be made of their services."

I think that every community that can carry a nutrition council should have one, and that this council should appoint a technical panel or team consisting of persons from the departments of health, agronomy, education and administration. All members of the panel should possess special knowledge of nutrition and should have had field experience of both survey and development work in nutrition, in relation to their respective fields. It will probably be found necessary, in addition, to employ someone whole-time on nutrition work to act as co-ordinator of this body and its work, in council and in the field. I know of no better training for a team for this kind of work than to undertake a year's co-ordinated survey of the kind made under my direction in Nyasaland. By this means, I believe, the staff that will ultimately have to accept responsibility for the improvement of nutrition will, at the very outset, secure an appraisal of their nutrition problems which, with occasional expert assistance and advice, will enable them to plan and carry out plans. Their efforts should ultimately become an integral part of the routine work of their respective departments.

Research in the Future

What are the lines along which research in nutrition is likely to develop in the future? First, there will be day-to-day problems in medicine, food technology, sociology and economics associated with the formulation of nutrition policies. For example, in the Human Nutrition Research Unit, in food technology we have been working on a method of drying foodstuffs suitable for use on a small scale and in which food values and flavours are well conserved. In the medical field, work being done on the effects of nutritional and environmental factors on the healing of skin-wounds constitutes an attack on the problem of tropical ulcer. We have also explored the microbiology of the production of native African beers.

In general, however, the tendency will be for fundamental work on nutrition to be in the associated sciences. There is a great deal to be done before we understand the function of the various nutrients in the body; no doubt in this connection the employment of new techniques, such as the use of marked atoms, will prove of great value. Again, the exploration of microbiological processes in nutrition of man and animals has scarcely begun, while our knowledge of plant physiology and biochemistry is scarcely more than rudimentary, and advances in these sciences must precede further significant advances in the chemistry of foodstuffs and their production. There will probably prove to be an important relationship between nutrition and the resistance to and recovery from zymotic disease of all kinds—a field hardly recognized. The interrelationship of the utilization of nutrients, and the production and function of endogenous substances such as the hormones, should also be further explored. Lastly, when there is no longer work to be done on the pathology of malnutrition, there is a field as wide as physiology itself in determining the effects of the supply of nutrients on the integration of physiological processes.

Conclusion

Although this outline of past and present trends of nutritional research and practice is an expression of the personal views and experiences of one worker, it reflects also the general development of the subject. At first the emphasis was on the study of major nutritional disorders. This conception of nutritional science as the identification and study of disease due to gross nutritional deficiency has now evolved through several stages to a deliberate planning of human society to ensure optimal nutrition. The beginnings of nutritional research were in medicine, agronomy, and the related sciences, and it seems probable that the present period of nutritional specialism will ultimately have served its purpose and will be followed by a resumption of the former division of responsibilities.

The practice of nutrition is now undergoing a phase of activity and popular interest. As newly acquired knowledge of microbiology at one time dominated various aspects of public-health work and claimed special attention in industry, so the practical application of nutritional knowledge is prominent in the present phase of development. In the course of time, however, with continued demonstration of the benefits which must accrue from world-wide attainment of good nutrition, with ever-widening diffusion of knowledge of its principles, and with trained personnel working under the ægis of responsible agencies, the practice of nutrition will in its turn cease to occupy so prominent a position and will emerge into the ordinary routine work of these agencies.

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[1] [*see BMB* **493**]

PROTEIN METABOLISM

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The organic matrix of the body is essentially protein in nature and protein also plays an important part in the osmotic relationships of the body fluids and in the nutrition and regulation of the cells which these fluids bathe. Although seeming to play a static role, there is nevertheless in this matrix a dynamic equilibrium between the protein constituents and the amino-acids which are present in the body fluids.

The number of proteins is very large, yet they are all constructed of relatively few units—the amino-acids—whose number is about equal to the letters of the alphabet, but whose position and preponderance in the protein varies with each protein and gives to it its peculiar properties. These amino-acids have an extraordinary facility for being joined in chains, as it were head to tail, by means of peptide linkages. These chains, and there may be as many as 16 component chains, seem usually to be rolled up so as to take on a globular or ellipsoidal shape: exceptions are the long fibrous proteins like hair.

The process of digestion provides the organism with the building stones—*Bausteine*—for the creation of its own tissue proteins. The products of enzymic hydrolysis do not necessarily conform to the body's requirements but further transmutations take place to meet this defect and also to meet the requirements of the moment. Two of these will first be considered.

Protein Transmutation

Transamination: Protein has been said to contain the magic of life: ever newly created, ever dying. The truth of this has received considerable emphasis from the work of the two Russian biochemists, Braunstein & Kritzman (1937). They described this process of " transamination ", which is a shuttle mechanism that enables the cells to change keto-acids to amino-acids by replacing the oxygen atom of the ketone with NH_3 transferred from the $CH(NH_2)$ of either of the dicarboxylic acids, aspartic or glutamic, to the CO group of the ketonic acid. By partial oxidation of carbohydrates, some at least of the keto-acids can be produced, and in the process of transamination we have a means by which the body can form a portion of its amino-acids. The muscles are particularly rich in the enzyme which brings this about. An excellent review of this special mechanism has been published by Cohen (1942).

Transmethylation: It was du Vigneaud and his co-workers (1939, 1940) who first postulated and later gave direct proof that methyl groups can be transferred from methionine to other substances in the body. They identified choline in the vitamin-B complex as being capable of being formed from methionine with the aid of methyl groups. They administered methionine of which the methyl group was marked by having one of its hydrogen atoms replaced by deuterium. When this " labelled " methionine was fed to rats on a choline-free diet, choline could be isolated from their tissues with part of its methyl groups containing the deuterium. Du Vigneaud, Cohn, Chandler, Schenck & Simmonds (1941) also found that this marked methyl group could be transferred from choline to creatine. The methyl group of methionine could therefore be used by the body to synthesize two of its essential non-amino-nitrogenous constituents. The transfer of methyl groups from methionine to creatine was demonstrated by Borsook & Dubnoff (1940).

The brilliant work of the late Dr. Schoenheimer and his colleagues over a number of years has, more than anything else, demonstrated the extent to which nitrogen fed in the form of amino-acids is synthesized into tissue proteins, both in the form of the fed amino-acids and of other amino-acids to which the nitrogen is transferred by transamination. In these experiments they have used heavy nitrogen (N^{15}), deuterium and radioactive carbon. They have found that every protein in the body is continually changing and renewing its structure. By the use of these isotopes they have shed new light on some of the most hidden mysteries of intermediary metabolism. (In 1942 Schoenheimer and Ratner reviewed their work in relation to protein metabolism. Numerous papers from Schoenheimer's department have appeared since in the *Journal of Biological Chemistry*.)

In many other directions considerable progress has recently been made in our understanding of the physiology and pathology of protein metabolism. Some of these will now be considered.

Protein and Amino-Acid Requirements

The main function of protein in the diet is the provision of nitrogen and sulphur in the form of amino-acids for growth, maintenance, reproduction, lactation, repair and convalescence. Some proteins are also a valuable source of phosphorus. In recent years, with improved analytical methods, the centre of interest has been shifting from the proteins themselves to their constituent amino-acids, and the urgent problem in this field is the determination of the amino-acid requirements for man in health and disease.

The task of finding which of the 22 amino-acids are indispensable parts of the diet, and which the animal body can build for itself, has exercised the attention of many leading biochemists. The names of Hopkins, Mendel, Osborne and Rose are especially associated with this field of work. Rose (1938) was able to divide the amino-acids into two groups according to whether they need or need not be supplied in the diet of the growing rat. The amino-acids which were found to be indispensable in the diet were lysine, tryptophane, phenylalanine, leucine, isoleucine, threonine, methionine, valine, histidine and arginine.

These results Rose afterwards confirmed in the dog, and later Bauer & Berg (1943) confirmed them in the mouse. The qualitative requirements having been worked out, further tests demonstrated the approximate percentage of each of these amino-acids which must be included in the diet to produce the maximum rate of growth of the rat.

For the maintenance of nitrogen equilibrium, histidine and arginine did not appear to be essential. Methionine, if sub-optimal in amount, could be supplemented by cystine for growth-promotion, and the same relationship might exist between phenylalanine and tyrosine.

Burroughs, Burroughs & Mitchell (1940) and Mitchell (1942), from a study of the maintenance of the adult rat, came to the conclusion that lysine, leucine, histidine and arginine were not necessary and that tyrosine could apparently replace phenylalanine. Norleucine appeared to be able to function by promoting the synthesis of lysine or leucine or both. These workers came to the conclusion that for maintaining nitrogen balance in the adult rat the following amino-acids were required: threonine, isoleucine, leucine or norleucine, tryptophane, valine, and methionine. The authors consider that only in growth does *total* protein synthesis occur, while in the adult animal only " particular requirements " must be met.

Within the last year or two, Rose has been able to accumulate sufficient of the amino-acids found essential for the growing rat to make human trials possible. No one has yet ventured to determine the growth requirements of the child, but studies on adult men are now in progress. So far, it appears probable that what was found necessary for the rat will also be found qualitatively necessary for the human, and the non-essentiality of the 12 amino-acids which were found dispensable for the rat applies also in man. The essentiality of valine, without which there is a profound and immediate negative balance, methionine, threonine, leucine, isoleucine, and phenylalanine, have already been confirmed. Removal of histidine produced no disturbance of nitrogen equilibrium, indicating its apparent non-essentiality for maintenance (Rose, Haines, Johnson & Warner, 1943). Albanese *et al.* (1941, 1943a, b) and Holt *et al.* (1941, 1942) have also studied the effects of the following deficiencies in man: tryptophane, lysine, arginine, methionine and cystine. They found that when arginine was removed from the diet a reduction in the number of spermatozoa in the seminal plasma took place.

Madden, Carter, Kattus, Miller & Whipple (1943) have found that the 10 amino-acids found adequate for growth when tested on standardized plasma-depleted dogs produced new plasma protein in abundance. It was found that methionine could not be replaced by cystine without affecting the other tissues. Although not required for nitrogen balance for as long as 1 to 2 weeks, arginine was required in the diet of the plasma-depleted dog for plasma-protein formation. It is of interest that the glycine requirements of the chick are so high that glycine becomes an essential amino-acid (Almquist, 1942).

Attempts have recently been made to determine the specific effects of single amino-acid deficiencies. The work of Harris, Neuberger & Sanger (1943) indicates that as the result of lysine deficiency cessation of growth and hypoproteinæmia develop in young rats. The number of erythrocytes and the amount of hæmoglobin per unit volume of blood are slightly lower than in the control animals. These changes are believed to indicate a retarded development of the hæmopoietic system. Radiologically the deficient animals exhibit a decrease in subcutaneous fat, muscle-wasting, and reduction of calcification in the bones. The epiphyseal cartilage in the long bones is barely visible and there is histological evidence of a reduction in the number of chondroblasts in the first zone of proliferating cartilage. Mitosis

appears to be reduced in the testes. This deficiency leads to a general inhibition of protein formation and protein is transferred, e.g. from muscle, according to a fixed system of priorities—the body-weight remaining fairly constant and the effect being similar to partial starvation.

One of the difficulties in testing the effects of deficiency is the effect that the deficiency often has on appetite. Paired feeding controls eliminate this difficulty to some extent, although it may be argued that any decline in appetite is part of the symptom-complex of the deficiency.

Albanese & Irby (1943) have brought forward some indirect and not very convincing evidence that the unnatural (*d*-) forms of certain of the amino-acids which cannot be utilized may have a toxic effect. Kinsey & Grant (1944) have found no evidence of this in the rat, in spite of using racemic mixtures of isoleucine, methionine, phenylalanine, threonine, tryptophane and valine, and no evidence of toxicity has emerged from human trials. The *d*- and *l*- forms of methionine are both utilized. The metabolic fate of the unnatural form of the others is largely unknown, but it is generally believed that they are not utilizable.

Methionine as a precursor of cystine plays a very important role in the formation of the scleroproteins of the epidermal structure and also of certain hormones, particularly insulin. Phenylalanine presumably furnishes the nucleus for the synthesis of thyroxine and of epinephrin. Arginine is considered to supply the amidine group for the synthesis of creatine (Block & Schoenheimer, 1941).

Melnick (1943) gives interesting data on the composition of the average daily American diet per capita in terms of essential amino-acids. As the methods of analysis for certain amino-acids are still very difficult and uncertain, too much stress should not be laid on the accuracy of these figures which are: lysine, 5·1 g.; tryptophane, 1·1 g.; phenylalanine,[1] 4·3 g. (tyrosine, 3·8 g.); leucine, 10·7 g.; isoleucine, 3·3 g.; threonine, 3·4 g.; methionine,[1] 2·8 g. (cystine, 1·2 g.); valine, 4·2 g.; histidine, 1·8 g. and arginine, 4·8 g. This accounts for about half the total amino-acids in the diet.

Block & Bolling (1944) have also discussed the amino-acid constitution of different proteins and their ability to furnish the essential amino-acids, and the following salient points are taken from their paper.

Human milk proteins yield significantly more arginine, tryptophane and cystine plus methionine than cow's milk. Human milk appears also to be richer in isoleucine. It is considered that the proteins in milk are so constituted that the infant can form the maximum quantity of new tissue from the minimal amount of ingested protein.

It is suggested that the best way to enable the organism to replenish its blood proteins after extensive loss would be to supply histidine, lysine and threonine, as they are high in blood proteins when calculated on the basis of two-thirds hæmoglobin to one-third serum proteins. Blood proteins have low contents of cystine, methionine, and isoleucine.

Egg proteins are decidedly higher in arginine and the S-amino-acids than milk and blood. The formation of these proteins is probably concerned with the high requirement of the growing chick for these amino-acids.

There do not seem to be any marked differences between the meat- and fish-muscle proteins, with the possible exception of the lysine content, which may be significantly higher in meat proteins. Meat yields more lysine but less tryptophane and cystine than does egg. The small quantity of these last two amino-acids in meat proteins may account for their lower nutritive value as compared with egg proteins. Meat is a superior source of arginine, methionine and probably cystine but inferior as a source of the aromatic acids. Gelatin is deficient in, or almost devoid of, histidine, tyrosine, tryptophane, phenylalanine, cystine, methionine, threonine, leucine, isoleucine and valine.

Maize (corn) proteins may be richer in tyrosine, methionine, leucine and valine but poorer in tryptophane and possibly in phenylalanine than those of wheat. With regard to the proteins of the germ or embryo, those of maize appear to be a better source of all the aromatic amino-acids as well as of those of the leucine group. Both maize- and wheat-grown proteins are well balanced with respect to their essential amino-acids and compare favourably with the common proteins of animal origin. The nutritive values of milk and maize proteins are approximately equivalent for purposes of growth, and the addition of either to the proteins of refined wheat flour resulted in a marked increase in the growth-promoting ability of the diet. Maize gluten yields less histidine, lysine, tryptophane, and glycine, but more tyrosine, methionine, leucine, isoleucine, and valine, than wheat gluten. The easiest way to improve the nutritive value of wheat gluten is to incorporate lysine or a lysine-concentrate into the flour.

Cottonseed, linseed, and peanut yield adequate quantities of arginine and histidine, but only one-third to one-half of the lysine furnished by most edible animal proteins. Linseed meal appears to be rich in tryptophane, being equal to or apparently better than milk. Cottonseed and peanut proteins are rather low in methionine and threonine, but higher in valine than any natural food considered in the paper under discussion.

Although yeasts appear to furnish proteins superior even to soya beans, the balance of the essential amino-acids in both these foodstuffs is generally satisfactory, except for the mild deficiency in methionine. By the inclusion of whole corn or corn gluten in the diet, or possibly the addition of a small quantity of cystine, this lack can be corrected, as the lecithin present in whole soya beans should provide an ample quantity of methyl groups in the form of choline.

Meats and fish furnish half the lysine intake even though they account for only 38 % of the total protein. Meats and fish are also superior sources of arginine and threonine. The presence of collagen in meat will tend to decrease cystine, methionine, tyrosine, tryptophane and threonine, and increase arginine and especially glycine.

If the freely selected mixed diet in America during 1937–41 be taken as representing a suitable intake of amino-acids, then eggs are the only single article of diet which supply the essential amino-acids in truly balanced proportions.

It does not appear that soya beans, peanuts, etc. can fill the void created by the marked deficiency in lysine of the cereal grains. Although cereals are consumed to the extent of 27 % of the total protein of the diet they furnish only 10 % of the lysine.

Studies on a low economic group made in Spain (Robinson, Janney & Grande Covián, 1941) have shown that in spite of an apparently adequate intake of total protein (66 $\pm$ 2·7 g. per day) and of iron (11·4 $\pm$ 0·43 mg. per day), one-sixth of the group exhibited dyscrasias which may possibly have been directly attributable to a deficient consumption of lysine since the intake of animal protein was 22 $\pm$1·7 g.

If the sole source of energy and protein were bread, it would be necessary to consume approximately 6½ pounds [about 3 kg.] daily for optimal amino-acid intake. However, if either (*a*) 2 g. lysine per 100 g. protein is added to bread made with 6½ % milk solids, or (*b*) 3 g. lysine per 100 g. protein is added to bread without milk solids, the average daily requirement for the essential amino-acids would be met by consumption of only 108 g. protein in the form of approximately 3,600 calories (equivalent to slightly more than 2½ pounds of bread).

A few years before the war Boas Fixsen (1935) reviewed the whole field of the biological values of proteins. During the war, British work on this subject has naturally turned to the proteins of wheat flour submitted to different degrees of extraction and also to previously unexplored sources of protein, in the hope that some unusual protein of high biological value might come to light which would compensate for the shrinkage in animal protein which appeared imminent.

Chick (1942) found that the proteins of *National Wheatmeal* (85%-extraction) flour had an advantage over the proteins of white flour (75 % extraction) of 13 % to 16 % extraction, and that the biological value of the proteins of whole wheatmeal was even higher, namely 17 % to 26 %. The calculated coefficients of digestibility of the proteins of wholemeal and *National Wheatmeal* flours were lower than that of white flour by 6 % and 3 % respectively, losses which were more than compensated by the greater nutritive value of these flours.

It has recently been found that the non-protein nitrogenous constituents of expressed potato juice contain some substance(s) which so complement the amino-acids of the protein of the potato as to produce a mixture of biological value equal to that of the protein itself (Chick & Cutting, 1943).

[1] [Phenylalanine is the essential amino-acid from which the body can form tyrosine. As tyrosine is required for certain specific purposes—e.g. thyroxine synthesis—the presence of dietary tyrosine reduces the dietary requirement of phenylalanine. There is a similar relation between methionine and cystine.]

Pirie (1942) has suggested that under certain circumstances, the conversion of part of the protein of leaves into human food could be more effectively performed by industrial chemical methods and equipment than through the ruminant. Although adequate feeding experiments with leaf proteins have not yet been made, the available data on amino-acid composition and early feeding experiments with dried leaves suggest that such a protein is of high biological value. Grass, lucerne and cabbage are considered the best sources. It is estimated that 200 pounds [about 90 kg.] of leaf-protein N could be harvested from one acre [about 4,000 m.²] each year, and Pirie has suggested that research should be devoted to the maintenance of a supply of leaf material of high N content during several months of the year, and to the design of a suitable macerating plant.

As revealed by the Report on Food Consumption Levels, the protein available per head per day of the civilian population in the United Kingdom did not fall below 80 g. (36 g. animal) even in 1940 (Ministry of Food, 1944).

Interest has been developing in the determination of the biological values of the proteins of a mixed dietary and Macrae, Henry & Kon (1943) have recently determined the biological value of the mixed proteins of the diet served at four *Royal Air Force* stations, as determined by Mitchell's method. They found values of 77·7 to 81·2—which are surpassed only by the proteins of eggs and milk. This observation indicates that the process of amino-acid supplementation which takes place in a normal diet is effective in compensating for the varying deficiencies of the individual proteins composing it.

Even non-essential amino-acids and incomplete proteins have a nutritional value, and the latter may supplement the value of proteins of higher biological efficiency than they themselves possess. Although much work still remains to be done in assessing the biological values of the mixed proteins of different protein-containing foodstuffs, more attention must be directed to determining the amino-acid composition of these proteins. Once this is accomplished and our requirements for the essential amino-acids are known, it will be possible to predict the biological value of a protein or mixture of proteins from its amino-acid constitution.

Microbiological assay methods for amino-acids: The discovery by Mueller & Cohen (1937) that β-alanine is an essential growth factor for the diphtheria bacillus has provided a microbiological assay method for this compound. Recently a number of papers have appeared dealing with the microbiological determination of amino-acids using cultures of *Lactobacillus arabinosus* (Kuiken, Norman, Lyman & Hale, 1943; Shankman, Dunn & Rubin, 1943a, 1943b; McMahan & Snell, 1944) and of *Lactobacillus casei* (Shankman, Dunn & Rubin, 1943b; McMahan & Snell, 1944). By omitting one of the amino-acids essential for the growth of these organisms, a medium for the estimation of that particular amino-acid is prepared, titration of the amount of lactic acid formed in the test culture being indicative of the amino-acid which is present in the unknown. This method is useful for estimating the presence of some 11 amino-acids in hydrolysates from less than 1 mg. protein, including valine, leucine and isoleucine, which are difficult to estimate chemically.

Protein in relation to mineral metabolism: McCance, Widdowson & Lehmann (1942) have recently reported studies which show that in the adult subject periods of high protein intake induced high net absorptions of calcium and high urinary calcium outputs. The retentions of magnesium were greater in the high protein period and again there was a higher urinary output as compared with a low protein diet. There was also evidence of a slight beneficial effect on phosphorus but little effect on iron absorption. It is suggested by the authors that very little calcium would be absorbed if the diet contained no protein or amino-acids.

Linneweh & Shobin (1940) found evidence in plasma-depleted dogs that there occurred a definite, simultaneous, and parallel reduction in total protein, albumin and globulin and in the total and ultrafiltrable calcium. After intravenous administration of calcium, either the serum calcium and the protein levels were raised or the fall was prevented, again in parallel. In cases of hunger-œdema, eczema and severe anæmia studied, the calcium level of the blood rose with the protein level. These workers therefore concluded that the calcium level of the blood is not entirely regulated by hormones or vitamins, but that it is, partially at least, a function of the protein level.

Quantitative requirements of protein: For the more quantitative aspects of protein nutrition the reader is referred to the reviews which appeared in *Nutrition Abstracts and Reviews* during the period 1936–40, by Garry & Stiven (1936) on the requirements in pregnancy and lactation, by Leitch & Duckworth (1937) on the determination of the protein requirement, and by Cuthbertson (1940) on the quality and quantity of protein in relation to health and disease. Since its inception in 1932 there have been admirable summaries on the biochemistry of the proteins and the amino-acids, and also on nutrition, in *Annual Review of Biochemistry*.

In 1941 the Committee on Food and Nutrition of the U.S.A. *National Research Council* recommended daily allowances of protein according to age, sex and level of activity. The writer has revised these in terms to suit the dietary habits in the United Kingdom (Cuthbertson, 1944a) [see table].

TABLE

COMPARISON OF AMERICAN ALLOWANCES OF CALORIES AND PROTEIN (N.R.C. STANDARDS) WITH VALUES REVISED TO SUIT DIETARY HABITS IN THE UNITED KINGDOM

	American allowances			Revised (net values)		
	calories	protein g.	% of calories derived from protein	calories	protein g.	% of calories derived from protein
	70 kg.			61 kg.		
Man						
moderately active	3000	70	9·6	2900	75	10·6
very active	4500	70	6·4	4200	110	10·7
sedentary	2500	70	11·5	2400	65	11·1
Woman (56 kg.)						
moderately active	2500	60	9·8	2500	65	10·7
very active	3000	60	8·2	3200	80	10·3
sedentary	2100	60	11·7	2100	55	10·7
Pregnancy (latter half)	2500	85	13·9	2500	75	12·3
Lactation	3000	100	13·7	3000	95	13·0
Children up to 12 years						
1 to 3 years	1200	40	13·7	1200	40	13·7
4 to 6 years	1600	50	12·8	1600	50	12·8
7 to 9 years	2000	60	12·3	2000	65	13·3
10 to 12 years	2500	70	11·5	2500	80	13·1
Children over 12 years						
girls 13–15 years	2800	80	11·7	2800	90	13·2
girls 16–20 years	2400	75	12·8	2400	70	12·0
boys 13–15 years	3200	85	10·9	3200	100	12·8
boys 16–20 years	3800	100	10·8	3800	110	11·9

Protein Intake in Relation to Energy Expenditure, Muscle Work, Physique and Resistance to Disease

Energy expenditure: The present writer, as the result of an earlier survey of the literature (Cuthbertson, 1940), came to the following conclusions:

i. In its capacity to supply energy, protein is apparently no more useful than carbohydrate or fat, except by virtue of its specific dynamic action which makes it particularly suitable for those exposed to cold. This action, and other evidence as to the economic bodily use and storage of protein, strongly suggest that the digestion of protein should not be dissociated in time from the digestion of carbohydrate. The more perfectly balanced a diet, the greater is the efficiency with which protein is utilized and the less will be the wastage as heat.

ii. Man can apparently adjust himself to greatly varying levels of protein intake, and the protein may be exclusively animal or vegetable in origin, or of any intermediate mixture, no deleterious effects resulting from either extreme, provided the process of amino-acid supplementation is effective to cover the requirements of the individual essential amino-acids, and the total intake is adequate.

iii. The level of protein intake for any particular population, when expressed as a percentage of the total calories,

tends to remain constant at all grades of muscular activity with, perhaps, the exception of athletes training for special events, who in general select diets containing a higher proportion of protein, chiefly of animal origin. It is not clear what purpose this increased protein intake serves. Unless the diet contains a very high proportion of sugar, highly processed cereals, and fat, it is difficult to plan a diet providing less than 10 % of its calories as protein. Most diets, with the exception of those of some highly carnivorous races and tribes, have 10–14 % of their calories as protein.

iv. In general, it may be said that whenever and wherever economic circumstances permit, man elects to raise his intake of animal protein to the region of 60 % of the total protein.

Howe has recently (1942) provided details of the optimal dietaries planned for the U.S. soldier in training. These contain 120 g. protein and some 4,000 or more calories. This protein value works out at 12 % of the calories.

Muscle work : On the difficult question of the role of protein in muscle work, Gemmill (1942) has concluded from his survey of relevant literature that the use of carbohydrate is of primary importance, but that long-continued work produces an increase in the output of nitrogen in the urine which, however, bears no direct relationship to the work done. It is postulated that when exercise reduces the carbohydrate supply of the body, extra protein is deaminized to supply carbohydrate or carbohydrate intermediates. The evidence for this assumption is based on the observation that ingestion of carbohydrate abolishes the increment of nitrogen output. This viewpoint is in agreement with the earlier evidence of the present writer and his colleagues (Cuthbertson, McGirr & Munro, 1937).

Resistance to fatigue : The history of the use of glycine and gelatin (which contains 25 % glycine) in the treatment of muscular dystrophy and in relation to resistance to fatigue has recently been reviewed by King, McCaleb, Kennedy & Klumpp (1942), Beard (1943) and Keys (1943). The evidence is very conflicting and the case for glycine, on the balance of evidence, is " not proven." Beard believes that to demonstrate the effect of glycine it must be given in adequate amounts, e.g. 12 g. per day on a normal or high protein diet, and must be given for well over three weeks, as he found that the first increases occur after that period. He considers that if enhanced muscular performance has already occurred as a result of training, it is impossible to expect any additional substance to increase the work output.

Physique : From a survey of the diets of the peoples of the earth, it appears, in general, that those races having a fairly high protein intake, especially animal protein, are more virile, robust and energetic and have better physiques. It also appears that there is less active tuberculosis in races or groups with such a diet, but it is not proved that the increased resistance or physical well-being is due to protein itself or to an accompanying superior diet (Cuthbertson, 1940).

Immunity : In the field of immunity the evidence is fairly conclusive that an antibody is a molecule of globulin which, during synthesis, has been specifically modified under the influence of antigen. By the use of isotopic amino-acids it was found that the uptake of dietary nitrogen which accompanies body-protein synthesis, through amino-acid replacement and nitrogen transfer among individual amino-acids, was also shared by the antibody protein which is formed when isotopic amino-acids are administered to actively immune rats and rabbits (Schoenheimer *et al.*, 1942b). Antibody formation during active immunity appears to be a continuous process of production and shares in " labelled " N^{15} which has been added to the diet. On the other hand, passive antibody formation during passive immunity appears to have little relation to dietary N (Heidelberger *et al.*, 1942).

How far the antibody formation plays a really significant rôle as a competitor for the available amino-acids in protein under-nutrition is perhaps difficult to conceive, as the total amount required in proportion to the tissue proteins must be very small indeed. Nevertheless, probably just because there is so much competition for the available amino-acids in the under-nourished subject, antibody formation may share in the general restriction in protein anabolism.

Attention may also be drawn to the well-known tendency of marasmic infants to die of intercurrent infection, the great susceptibility of patients with nutritional œdema to respiratory and other infection, the terminal infections in malignant renal or hepatic diseases, and the general rise in death rates from tuberculosis and other infectious diseases in war time. The concomitant occurrence of hypoproteinæmia and an increased susceptibility to infection suggests the possibility of a mutual relationship. Animals made hypoproteinæmic manifest a definite loss of ability to produce antibodies of several kinds (agglutinins, precipitins, hæmolysins). The loss of acquired immunity is manifested particularly in diseases characterized by a marked protein deficiency. This aspect of the literature has recently been reviewed by Cannon (1944).

Nuclear and Cytoplasmic Proteins

The recent work on the importance of the nucleic acids and nucleoproteins in animal tissues, particularly in connection with cellular activity, has suggested that nucleoproteins containing pentose nucleic acids (" ribonucleoproteins ") play a special rôle in protein synthesis and tissue growth. It is now known that the nucleoproteins containing these nucleic acids are constituents of many self-reproducing filterable viruses, and they appear to be abundant in the rapidly growing cells of embryos, tumours, and regenerating tissues. Mirsky (1943) and Davidson & Waymouth (1944c), who themselves have contributed much of value in this field, have recently reviewed the evidence for the essential rôle of nucleic acids in nuclear structure and for the association of nucleoprotein and nucleic acids with the processes of growth. The evidence suggests that rapidly-growing embryonic tissues are characterized by both a high nuclear desoxyribopolynucleotide content and a high cytoplasmic ribopolynucleotide content (Davidson & Waymouth, 1943 ; 1944a ; 1944b).

A new conception of the chemistry of the nucleus has been opened by the discovery of a new nuclear protein " chromosomin " by Stedman & Stedman (1943a, 1943b, 1943c, 1944). This protein has predominantly acidic properties and is believed to be one of the principal components of the chromosomes.

Protein in Relation to the Function of Organs

Kidney : While over-eating in general seems definitely to be connected with arterial hypertension, there appears to be no real evidence for the belief that high-protein diets lead to nephritis and hypertension. Indeed, the general trend of work is to show the beneficial effects in some types of nephritis, particularly where œdema is present, and also in the toxæmias of pregnancy, of diets containing considerable amounts of protein daily. This high protein intake has a diuretic effect due to the amount of urea formed, and this assists in bringing œdema under control (Cuthbertson, 1940).

Recently attempts to test the effect of a high-protein diet on the organism with impaired renal efficiency, have been made on the dog by Philipsborn, Katz & Rodbard (1941). They found that no rise in blood-pressure resulted from the imposition of a high-protein diet in dogs with normal blood-pressure but with their renal arteries subjected to temporary total or chronic partial occlusion. Again, in animals with moderate hypertension and slight renal insufficiency, no rise was noted. It was only in some of the animals with moderate renal insufficiency and frank hypertension that an effect was observed. Guerrant, Scott & Wood (1943) also found that no consistent rise or fall in systolic or diastolic blood-pressure occurred in normal dogs, or in hypertensive dogs fed on a high-protein diet of lean raw meat for long or short periods, the hypertension being produced by partial occlusion of first one then the other of the renal arteries.

It has now been found that urinary protein is either entirely serum albumin or a mixture represented by the albumin : globulin ratio as found in the urine. The urinary protein of a nephritic was not altered by changing from an egg-free to an egg diet (Murrill, Block & Newburgh, 1940).

In Bright's disease, two changes of plasma amino-acid content in opposite directions have been noted in Van Slyke's laboratory (Van Slyke, 1942). One of them was discovered by Kirk in patients in or near uræmic coma. The plasma amino-acid level in some of these patients rose in a day or two from little above the normal level to six- or eight-fold as much. If recovery occurred, the plasma amino-acid level fell as quickly to normal. How far the sudden rise is due to an accelerated tissue-breakdown accompanied by a simultaneous failure of the liver to deaminize is a matter for conjecture, as the mechanism remains unknown.

In the opposite direction, Farr & MacFadyen (1940) found

that the plasma amino-acid level was below normal in the nephrotic type of Bright's disease and that recovery corresponded to a rise to normal levels. Acute clinical manifestations, "nephrotic crises," became apparent when the level fell to 2·5 mg. or lower per 100 ml. When 33–57 % of the dietary nitrogen of nephrotic children was replaced by a casein hydrolysate given intravenously, neither the course of the nephrotic disease nor its ultimate outcome was affected. For this reason the use of such a hydrolysate to treat the hypoproteinæmia of the nephrotic is not advocated by the authors. It is believed, however, that a positive bloodstream infection in nephrotic patients is a definite indication for intravenous amino-acid therapy.

It is not proposed to discuss here the rather academic researches which relate kidney hypertrophy to the presence of the excess of certain amino-acids in the diet.

Liver: A discussion on the relation of the liver to protein metabolism would occupy a monograph by itself. There are, however, certain aspects which are worth noting in passing. The first relates to the inter-relationship of certain amino-acids to the nutritive condition of the liver, methionine and cysteine in particular.

Miller & Whipple (1942) have recently demonstrated in the protein-depleted dog that methionine, if given shortly before chloroform anæsthesia, provides complete protection against hepatic damage, and that this amino-acid, or cysteine + choline, gives significant protection even if administered 3 to 4 hours after chloroform anæsthesia. After 4 hours no protection was provided. Choline itself did not give protection beforehand. The livers of these protein-depleted animals were deficient in both N and S, the latter in particular. Administration of methionine or cystine promptly made good this deficit.

These investigators, on the basis of this protective action of methionine (e.g. casein), or of various proteins by mouth or vein, suggest their use as therapeutic agents where there is any type of damage to the liver or other organs. Methionine in solution can be administered parenterally or with glucose, without any unfavourable reaction in man and animals. It had previously been suggested by Miller, Ross & Whipple (1940) that chloroform, by combining with the sulphydryl groups, may inactivate enzyme systems unless there is an adequate reserve of these groups.

Supplements of methionine, cystine or cysteine, especially the last, largely prevented the marked inhibition of growth in young rats fed on an 18 % casein diet containing 0·12 % cobaltous sulphate. The high mortality due to oral or intraperitoneal administration of very toxic levels of cobalt or nickel was also prevented by the simultaneous and separate administration of cysteine. The complex formed between cobalt and cysteine *in vitro* was relatively non-toxic, suggesting that cobalt poisoning is due to the fixation and loss of sulphydryl compounds in the tissues.

Messinger & Hawkins (1940) found that protein was the most effective dietary principle in protecting dogs against arsphenamine liver injury. On a protein diet the damage was trivial and was rapidly repaired. Carbohydrate was not uniformly so successful: fat was definitely deleterious to the dogs.

Probably even more important than its effect on susceptibility to known toxic agents, is the direct necrotizing effect on the liver of methionine deficiency alone. Depending upon the degree of the deficiency, changes in the rat's liver ranging from fatal acute yellow atrophy to a variety of chronic hepatic cirrhosis have been produced by Himsworth & Glynn (1944) in animals given a low-protein diet. The determining factor is the amount, quality and quantity of protein eaten. Casein, even in small amounts, protects against the lesion, while yeast protein even in liberal amounts fails to protect. These investigators have found that complete protection can be provided by methionine—an amino-acid in which casein is particularly rich. They put forward reasons for believing that, in man, massive hepatic necrosis and its sequel nodular hyperplasia may be attributed to a trophopathic hepatitis, and that, whatever the nature of the primary illness, this massive hepatic necrosis is to be regarded as a conditioned deficiency disease. The action of many so-called liver poisons, e.g. trinitrotoluol, cinchophen and neoarsphenamine, is believed to be brought about by their interference with methionine in the body.

Beattie & Marshall (1944) have published their preliminary results based on a study of 450 cases of infective hepatitis and "post-arsphenamine" jaundice. These would appear to indicate a beneficial effect of the methionine and of casein digests rich in methionine. "In gravely ill patients, the results obtained by methionine treatment have been so striking as to leave no doubt as to the efficiency of the treatment, especially in those cases which have remained jaundiced for weeks or months and were in a state of icterus gravis when methionine was initiated." The addition of cystine is believed by the authors to spare the methionine. That caution should be exercised before accepting their findings as revolutionizing treatment is evidenced by the note by Peters, Thompson, King, Williams & Nicol (1944), which appeared shortly after Beattie & Marshall's report. Peters *et al.* have now studied more than 450 cases of "post-arsphenamine" jaundice and have found that methionine and also cystine had a significant but not remarkable effect on the course of the jaundice. Casein, equivalent in methionine content to that administered separately, was found to have no action. This could not be explained. While it is quite conceivable that there may be a fixation of arsenic through sulphydryl compounds, there is as yet no reason to believe that these compounds should be of benefit in infective hepatitis, the ætiology of which is completely unknown.

Choline prevents and cures fatty liver in rats and for this reason was called a "lipotropic" substance. Deficiency of choline appears to lead to a deficiency of the compounds concerned in lipid transport, and renal hæmorrhage may occur at a critical period when there is an acute shortage of essential cellular materials. Betaine, methionine and other substances may also be regarded as lipotropic. For diets free of choline, betaine, methionine and other lipotropic factors, it is suggested that the term "alipotropic" should be used, and for the effects of cystine, cholesterol and other substances with similar action in this sense it is considered that the term "antilipotropic" would be appropriate.

Recent work on arsenocholine has shown that this compound behaves in a similar fashion to choline in its lipotropic action and in preventing and curing acute hæmorrhagic effects. Arsenocholine appears to be utilized intact in the biosynthesis of lecithin. It is completely inactive as a methyl donor, and this suggests that choline exerts its effects through reactions involving the intact molecule. Previous theories on the lipotropic activity of choline, betaine and methionine had been based on their possession of a labile methyl group. By using radio-active phosphorus and heavy nitrogen it has been shown that choline and other lipotropic factors accelerate the elimination of phospholipids in the liver and other tissues; the most active seat of change being the former.

Ethanolamine is the generally accepted precursor of choline. The choline content of the body is dependent to a limited extent only upon the amount of choline in the diet, the level being maintained by methylation. The source of liver fat in the alipotropic diets appears to be mainly dietary fat or dietary carbohydrate.

The anti-hæmorrhagic action of choline, betaine and methionine seems to parallel their lipotropic effect. Cystine and cholesterol may counteract both effects. The rate of phospholipid turnover in the kidney is accelerated by choline but to a less extent than occurs in the liver. The anti-hæmorrhagic action is probably general but varies in degree with the different tissues.

Choline naturally promotes the growth of young rats subsisting on a relatively alipotropic diet. It can also accelerate growth when added to an alipotropic diet containing homocystine. The latter is neither lipotropic nor growth-promoting. Methionine is produced from homocystine by utilization of the methyl groups of choline. Homocystine, however, is not a natural product.

The literature on this subject is considerable but has been reviewed by Best & Lucas (1943) who hold that some international group should decide whether to call choline simply a dietary factor (as the authors term it), or a vitamin (as György would term it), or a vitagen (according to Rosenberg). From the metabolic aspect it would appear appropriate to group it with the B-complex.

Hæmoglobin and the Plasma Proteins

It is well known that hæmoglobin production in anæmia can be controlled by diet—more specifically that iron and protein stores or intakes are essential factors. Hahn & Whipple (1939) have clearly demonstrated that, by limiting

the protein intake in anæmia, hæmoglobin production can be reduced. When the erythrocyte and its contents disintegrate, much of the globin is saved and is probably used again to form new hæmoglobin or to supply some other protein needs of the body. Hæmoglobin production is modified by infection and to some extent in the later stages of nephritis (Whipple, 1942). Robscheit-Robbins, Madden, Rowe, Turner & Whipple (1940) have found that in dogs given abundant iron, but bled almost daily to maintain an anæmia level of about one-third normal, two to three times as much hæmoglobin was produced as plasma proteins even when the stimulus was apparently maximal.

The source, production and utilization of the proteins of the plasma were reviewed in 1940 by Madden & Whipple and again in 1942 by Whipple. The evidence is clear that the liver is of primary importance in the production of plasma proteins, although some globulin may be produced elsewhere: there is in fact evidence that fibrinogen is wholly dependent upon liver function.

Per unit of protein fed, beef serum will favour the production of three times as much plasma protein as beef heart, and more than five times as much as beef stomach: there is thus a qualitative as well as a quantitative aspect to the influence of food protein. Casein and lactalbumin have also been reported by some as having high potencies; low potencies are recorded for spleen, erythrocytes, brain, canned salmon, gelatin, pancreas, kidney and zein.

Animal protein is twice as effective as vegetable in combating œdema and hypoproteinæmia. For albumin production, it is unsafe to make sweeping assertions concerning the group-superiority of animal or of plant proteins. However, certain plant proteins, e.g. rice and potato, appear to favour a low albumin: globulin ratio, and are favourable for the production of globulins when compared with certain standard animal proteins. The body appears to use both albumin and globulin at about the same rate to carry on its normal internal protein metabolism. Under the condition of plasmapheresis there are indications that globulin formation is directly dependent on the diet. Cystine, under certain conditions, appears to act as a key amino-acid in plasma-protein regeneration. Methionine is not apparently an efficient substitute for it. Parenteral liver extract and iron are ineffective in regeneration in hypoproteinæmic dogs.

There is considerable evidence that a reserve of plasma-protein-building material exists in the organism and may be supposed to be part of the body stores. In the utilization of the reserve store for general N requirements or for plasma-protein formation, a large bulk of the store is used early and rapidly. The "labile" portion may be regarded as merely that portion of the reserve store which is mobilized first and fastest. The greater bulk of this reserve is not stored as plasma protein, nor as material more easily converted into plasma protein than orally ingested protein. The store yields albumin on depletion in slightly more abundance than globulin. The reserve store of protein may be regarded as all protein which may be given up by an organ or tissue under uniform conditions without interfering with organ- or body-function.

Sachar, Horvitz & Elman (1942) have shown in dogs that there is apparently a constant relationship or partition in the loss (or gain) between plasma-albumin and the total body-protein induced by diet, and that this relationship is of the order of 1 to about 30. Thus, for every g. of increase in serum albumin desired, about 30 g. must be retained for increases in other proteins of the body. These workers stress that this relationship applies only to alterations induced by dietary changes and cannot be expected to hold in cases of hypoproteinæmia due to blood loss, burns, nephrosis or liver disease.

Further, owing to dehydration and consequent blood concentration, the serum-albumin concentration may not reflect the hypoalbuminæmia of depleted patients. This is an important point which is frequently neglected. It was also found that when dogs rendered hypoalbuminæmic by a three weeks' fast were fed meat *ad libitum* the albumin appeared to reach a static level more rapidly than did the globulin.

Schoenheimer *et al.* (1942a), by the use of isotopic amino-acids fed to rats, have found that the plasma proteins share in the metabolic reactions involving the incorporation of dietary nitrogen, that the various fractions share to about an equal extent, and that the rate of the process is approximately the same as that found in the kidney, liver and intestinal tract of the same animal. Parallel investigations on the erythrocytes showed that both the protein and the porphyrin of the hæmoglobin had a lower "chemical activity" than the plasma proteins. It may be that the anæmia which is a frequent consequence of severe injuries is due, in part, to the lower competition-factor which hæmoglobin and/or the erythrocytes seem to possess in comparison with the plasma proteins.

The plasma-protein-forming mechanism is disturbed by the complicated body reaction to infection. Prolonged hypoproteinæmia *per se* causes no damage to the protein-forming mechanism. A steady state of "ebb and flow" exists between the plasma protein and the cell- and tissue-protein. The ultimate source of the construction-materials is food protein, furnishing amino-acids which are synthesized in the liver cells (and elsewhere) into plasma proteins. This same influx of amino-acids and/or plasma proteins, supports protein formation throughout the body. A part of the body protein forms a reserve against adversity in the sense that it can be depleted without obvious injury to the body. It has been termed "the bulwark against infection".

Elman (1943), Wilensky (1944) and others have also reviewed the subject, more especially from the clinical viewpoint. Deficiency of plasma protein (hypoproteinæmia), including lessening of the normal reserve, occurs in conditions of deficient food intake (hunger-œdema), or of digestive capacity or absorption. It is frequent in bleeding peptic ulcer, carcinoma of the alimentary canal, intestinal obstruction and in patients with intestinal fistulæ. It is also found in liver diseases, and in forms of traumatic, toxic or chemical injury to the liver. In severe thyrotoxic disease, there is, in addition to the increased catabolism of protein, an associated parenchymal lesion of the liver. Hypoproteinæmia occurs also in renal conditions where albuminuria is an important factor. In certain severe conditions accompanied by excessive loss of nitrogen due to abnormal destruction of tissue, there are large losses of nitrogen in the urine, and the tissue and reserve stores of protein become abnormally depleted.

The general pathological changes due to hypoproteinæmia are those resulting from the disturbance in water distribution, namely, œdema of the subcutaneous tissues and of various viscera. Lesions of the tissues other than those associated with œdema commonly take the form of defects in protein tissue—partial disappearance of the cardiac muscle fibres and of skeletal muscle, fatty metamorphosis of the hepatic lobules with areas of necrosis in the mid-zonal areas, loss of substance and increased water content of the hepatic cell. The deleterious effect of hypoproteinæmia in the healing of wounds appears to be limited to the proliferation of the fibroblasts, but may well have more widespread effects.

Protein deficiency with anæmia occurs after severe injuries—particularly after extensive burns—following the actual loss of tissue, and of plasma in the exudate.

Reversal of the albumin: globulin ratio, which is normally 1·6 to 1, is found in advanced hepatic damage. Even where there is no reversal in such cases, the alpha, beta and gamma globulins are in abnormal distribution, the gamma form being present in increased proportion. In the hepatic type of hypoproteinæmia, the severe damage of the liver renders the low level of plasma protein relatively unresponsive to treatment, and the result depends largely on the length of time the hypoproteinæmia has been present, and on the amount of irreversible damage that has preceded the treatment. In most other forms of hypoproteinæmia, replacement therapy is usually effective, provided that the cause is removed. Replacement therapy may take the form of protein or amino-acids by mouth; transfusion of blood, plasma or serum; the reinfusion of ascitic fluid; and the parenteral administration of amino-acids alone or with blood, plasma or serum.

Kagan (1943) has pointed out that hyperglobulinæmia is chiefly found in diseases of the bone-marrow, chronic infection, diseases involving the liver, and dehydration. Hyperalbuminæmia rarely, if ever, occurs.

Feeding with Protein Hydrolysates and Mixtures of Pure Amino-Acids

When it was realized that extensive hydrolysis of protein was an essential preparation for the absorptive processes, and that amino-acids were absorbed from the intestine during digestion in amounts sufficient to account for all the nitrogen

digested, it was but a step to consider the administration of the split products themselves. Henriques & Andersen in 1913 demonstrated that nitrogen equilibrium could be maintained in the goat with intravenously injected amino-acids as the sole intake of nitrogen. The therapeutic implications of this did not follow until 26 years later, although the possibility of supplying amino-acids parenterally had been suggested from time to time, particularly by Rose (1934–35). In 1939, Elman & Weiner reported the application of a protein hydrolysate to the intravenous alimentation of human subjects. The preparation used was an acid hydrolysate of casein fortified with tryptophane (which is destroyed during digestion with acids), and supplemented with additional methionine or cystine, as they considered that the level of the sulphur-containing amino-acids in casein was not optimal for their purpose. Evidence of utilization, experimentally and clinically, as well as therapeutic effects in patients were observed by Elman & Weiner.

Cox & Mueller (1939) next reported that an enzymic hydrolysate of casein containing all the amino-acids of casein was capable of maintaining nitrogen balance and promoting growth. Clinical observations on this product by Shohl, Butler, Blackfan & McLachlan (1939) and by Farr & MacFadyen (1939, 1940) followed. The indications were that it was well utilized, but that reactions might be observed. With improved methods of preparation, the occurrence of reactions has almost completely disappeared, and thousands of intravenous infusions have now been given, particularly with enzymic hydrolysates of casein or meat. Casein digested by pork pancreas has largely been used in the United States by Elman and his co-workers and by many other workers there (literature reviewed by Martin & Thompson, 1943, and by Gaunt, 1944). Whole beef-serum has also been used (Melnick, 1943). A papain digest of casein, although it does not carry the digestion quite so far as trypsin, has been used experimentally by Madden, Zeldis, Hengerer, Miller, Rowe, Turner & Whipple (1942), and Beattie, Herbert, Wechtel & Steele (1944) have stated that they have used with success a hydrolysate of casein produced by submitting the protein first to the action of papain and then completing the digestion with trypsin. From India come interesting reports on the preparation and clinical use, in the Bengal famine, of papain digests of meat (Narayanan & Krishnan, 1944; Krishnan, Narayanan & Sankaran, 1944).

In order to prevent the amino-acids from being utilized mainly for energy purposes, it is always necessary to give carbohydrate simultaneously and, when they are given by vein, glucose is added to the hydrolysate. Hydrolysates are usually administered in 2·5 %, 5 % or 10 % strength.

It is obvious that there is no point in giving protein hydrolysates to patients who can ingest, digest and absorb sufficient protein to serve their requirements. It is only when the capacity to ingest, digest or absorb is affected that their administration is indicated. Orally, if suitably flavoured, they are a compact form of giving considerable amounts of amino-acids, particularly in conditions where appetite is lacking and the need for amino-acids is acute. Intravenously, they are useful as a temporary measure in supplying amino-acids in conditions where oral feeding is impossible or inadequate. Elman (1940) has reported a patient who had injections of 80 g. hydrolysate per day over 13 days. Taylor, Levenson, Davidson, Browder & Lund (1943b) have reported a patient who, as a result of extensive burns, became so desperately ill that excessive forced alimentation had to be attempted. This patient, fed by both vein and stomach tube, received some 500 g. of protein daily, of which up to nearly 200 g. were given by vein. This enforced feeding was maintained for more than 42 days and the patient is stated to have made a remarkable recovery.

In the Bengal famine of 1943, 25 % of the cases of inanition removed to hospital were almost moribund. They were unable to take even fluids by mouth. The immediate reaction to the glucose-hydrolysate mixture was usually good, and deaths due to inanition were reduced. In severe cases oral feeding was possible only after several injections. In some cases the improvement was not maintained and the patient died. At post-mortem examination, signs of serious complications were usually found; these were commonly indicative of dysentery, with extensive bowel ulcerations. The only contra-indication that Krishnan *et al.* (1944) have noted is nephritis. In such cases bad results have been seen. In liver conditions in which the deamination mechanism is impaired, as indicated by raised amino-acid N levels, and perhaps low blood-urea, the administration of large amounts of amino-acids would theoretically be harmful. Where, however, there is sufficient hepatic function, and yet because of ascites the patient is unable to ingest an adequate amount of protein, parenterally administered amino-acids may be useful.

Difficulties in the preparation of pure amino-acids have prevented more than a very few human experiments with them. At least two such experiments have now been reported (Shohl & Blackfan, 1940; Bassett, Woods, Shull & Madden, 1944). In the latter case the 10 essential amino-acids, together with some glycine, were given in 50-g. doses morning and afternoon over a considerable period of time. The rates of injection tolerated were greater than any recorded for protein-digest solutions. No rise in body temperature took place; 10 % glucose was also infused, and small oral feedings of carbohydrate and fat brought the daily caloric intake from all sources up to about 1,500 calories. Nitrogen equilibrium was achieved. It is of interest in view of the suggestion of Albanese & Irby (1943) that the *d*- forms of amino-acids might possibly be toxic, that the *d*- and *l*- forms of threonine, valine, leucine, isoleucine, phenylalanine and methionine were used without any evidence of toxicity.

The clinical indications for the use of protein hydrolysates and pure amino-acid mixtures have been discussed by Elman (1943), Elman & Lischer (1943), Martin & Thompson (1943) and Gaunt (1944). It is important to note that these hydrolysates or mixtures of amino-acids have not the colloidal properties of the blood proteins, and are in no real sense a blood-substitute, and that they should not be used routinely unless the ability to ingest, digest or absorb protein is seriously impaired. Further, it should be noted that, as at present arranged, only one or two proteins are administered in the hydrolysed form and their biological value will almost inevitably be less than that of the mixed proteins of a normal dietary. The indiscriminate use of these hydrolysates or artificial mixtures of amino-acids may lead to the discredit of a valuable therapeutic weapon.

Protein and Amino-Acids in Relation to Trauma and Wound-healing

The metabolic disturbance which results from injury can be divided into several phases. There is first the loss of tissue substance and the varying degrees of cellular damage at the site of injury. This represents an immediate loss of protein and the other cellular and intercellular substances. The anabolic activity of the living but partially-damaged cells in the neighbourhood of the lesion will be also disturbed. The amino-acid requirements to replace or heal the injured zone will naturally vary according to the nature of the tissue destroyed and the degree of fibrous repair. The more complex the tissue, the greater will be the proportion of fibrous tissue.

Depending on the severity of the wound and the loss of blood and/or plasma externally and into the tissue spaces, there will be a period of reduced blood volume and "oligæmic shock" may develop. There is thus a loss of protein from the circulating fluid which is probably only in part reversible. It would be out of place here to discuss the use of whole blood, plasma, serum, albumin, gelatin, isinglass, globin, etc., in the treatment of shock. It suffices to state that no substitute has yet been found which is as good as blood for the treatment of hæmorrhage, or plasma or serum to correct hæmoconcentration. Where damage to tissue exceeds in volume that represented by two closed or open hands, transfusion is usually indicated (Grant, 1944). Plasma and serum are frequently used in the treatment of hæmorrhage as they are more readily kept available, but when used in a volume exceeding two pints [about 1·14 l.] a careful watch should be kept on the hæmoglobin level or the erythrocyte count, so that whole blood may be given if anæmia develops (*Medical Research Council*, 1944).

It is only very recently that figures for the protein loss in the exudate of burns cases have become available and that their significance has been appreciated (Rossiter, 1943). Co Tui, Wright, Mulholland, Barcham & Breed (1944) have now found the loss of nitrogen in the exudate of a burned case to be at the rate of 0·42 mg./cm.^{2}/24 hours, and in another case whose surface had been denuded by avulsion, the rate was as much as 2·26 mg. of nitrogen/cm.^{2}/24 hours. These authors have calculated that for a 70 kg. man of 170 cm. in

height, a burn involving half the body surface would be equivalent to 23·75 g. of protein per 24 hours (= 400 cm.³ plasma or 114 g. lean meat) if the loss occurred at the rate found in the first case, whereas if it occurred at the same rate as in the second case, it would be equivalent to 124 g. protein per 24 hours (= 2,000 cm.³ plasma or 600 g. meat). The continued loss of plasma in the form of the exudate leads to oligæmia and this may cause " shock ".

During the period of " shock " the blood-flow to the kidney, liver, etc. is cut down to preserve the circulation to the more vital centres, and the function of these organs suffers in consequence.

Following this period of shock, with its lowered metabolic activity and with return of urinary flow to normal, there sometimes appear in the urine the products of the damaged tissues. This is mainly found in severe injuries, particularly crushing injuries. [Bywaters (1944) has given an account of these changes in air-raid casualties.] There is later a rise in metabolic activity which has been termed " traumatic inflammation." This precedes actual repair and is characterized by hyperæmia, exudation, and leucocytic emigration. During this period, autolysis and heterolysis through tissue and leucocytic enzymes remove the tissue debris; and sloughs which are not absorbed may separate.

Observations, mainly on fractures, have been made by the author and his colleagues (Cuthbertson *et al.*, 1929–44b) over a period of years and have demonstrated that, with this rise in metabolic activity, there is a marked loss of nitrogen, sulphur, phosphorus and potassium. Parallel with the rise in nitrogen, there is a rise in temperature and in oxygen consumption; creatinuria is also a feature. Disuse-atrophy, although it produces an undoubted but small loss of nitrogen, sulphur and phosphorus, could not wholly account for these changes, as the loss of substance was not confined to the area of injury (Cuthbertson, 1929; Cuthbertson, McGirr & Robertson, 1939). This traumatic catabolism in man usually reached a maximum towards the end of the first week after injury and later slowly declined, though a negative nitrogen balance might persist for several weeks after injury. With severe injuries, the ingestion of a diet rich in protein or in calories or in both, did not maintain nitrogen equilibrium at the height of the catabolic period, though there was evidence that with such high diets the nitrogen loss was diminished. How far this led to an improvement in the healing process could not be determined (Cuthbertson, 1936). In rats it was found possible to reduce markedly this nitrogen loss following trauma, e.g. fracture, by injecting the animals with a crude alkaline extract of the anterior pituitary gland which was already known to possess nitrogen-retention properties. On the other hand, there was no evidence from this, nor from wound-healing experiments, that the extract of the anterior pituitary gland accelerated wound-healing (Cuthbertson, Shaw & Young, 1941a; 1941b). On the other hand, evidence was gained that the healing of skin-wounds in rats could be accelerated by giving dried thyroid gland (Barclay, Cuthbertson & Isaacs, 1944).

Interest in this aspect of injury has received considerable attention recently in America and confirmation of many of these findings are to be found in the Macy Foundation Reports of the Bone and Wound Healing Conferences during 1942–43. Albright & Browne have there postulated that the adrenal cortex produces an " N " or nitrogen- or protein-anabolic hormone and an " S " or sugar or anti-anabolic hormone. These hormones are normally balanced, but after trauma has occurred there is an initial phase lasting 24 to 48 hours in which there is an increased excretion of both " N " and " S " hormones, followed by a second phase lasting days, weeks or even months in which there is decreased excretion of " N " hormone and an increased excretion of " S " hormone. Eventually, if the organism survives, the two hormones come into balance, and finally there may be a compensatory phase where the " N " hormone production is increased at the expense of the " S " hormone. Testosterone appears to fulfil the role of the " N " hormone. In the light of previous experience the writer doubts that testosterone therapy will achieve more success than that achieved by simply inducing nitrogen retention with anterior pituitary gland extract.

The theory has been advanced that the period of increased metabolic activity which is the concomitant of moderate or severe injury, might well be one of increased protein catabolism, involving the liberation of some essential key amino-acids for repair, and the oxidation of the others for energy purposes. In collaboration with H. N. Munro (Munro & Cuthbertson, 1943) the writer has found that when rats are injured (fracture) whilst subsisting on a protein-free diet, no disturbance of protein metabolism (traumatic catabolism), such as has been noted in normally fed controls, takes place, apart from a very slight rise in nitrogen excretion, probably due to the elimination of the products of muscle-trauma in the urine. This suggests that the protein catabolized is essentially derived from a labile reserve which could readily be exhausted. It has been shown (Cuthbertson *et al.*, 1937a, b, c) that with the ingestion of food in excess of that required to meet energy expenditure, there is built up a reserve of labile body-protein. Reference has already been made to Whipple's conception of a labile reserve (Whipple, 1938, 1942).

Somewhat the same changes were reported in experimental burns by Clark & Rossiter (1943), working in R. A. Peters' laboratory, but they are complicated in man by (*a*) the protein lost in the exudation from the " weeping " surface, especially of superficial burns, but present also during sloughing and the granulation period, (*b*) the effect of the coccal infections of the first week and the abundant mixed coccal and bacillary infections of the second to fourth weeks (deep burns) while the necrotic tissues are separating. The continued fever and chronic suppuration lead to a continued loss of tissue protein and to anæmia. Atrophy due to disuse and to reflex action is also considerable. Penicillin and other chemotherapeutic agents aid in suppressing losses due to infection. The extent of the loss of nitrogen has never been completely assessed, for data on the loss in the exudate have been lacking. The author calculates that, in the first ten days after a 60 % burn involving the whole thickness of skin, a patient may lose about 2 kg. of protein. The loss does not continue at this rate but gradually diminishes. Nevertheless the demands on the reparative process have to be met by a protein-depleted subject, and at a time when, by the very nature of the illness, appetite is reduced.

The recent observations by Lucido (1930), Browne (1942), Cope, Nathanson, Rourke & Wilson (1943), Taylor *et al.* (1943a, 1943b), Co Tui *et al.* (1944), Anderson & Semeneoff (1944) and others, have shown the extent of the protein loss in burns—a loss not fully appreciated until the recent figures of Co Tui *et al.* for the protein in the exudate became available. Previous estimations of the nitrogen balance are incomplete, and the apparent success in maintaining equilibrium as recorded by some observers may well be fallacious for the above reason. Co Tui and his colleagues have shown that, in the later stages, burned patients fed a diet high in calories and high in amino-acids, as administered in the form of a casein hydrolysate, could be kept in nitrogen balance. It is difficult to determine from the protocols how successfully a balance might have been achieved by giving more natural food. The administration of amino-acids in amounts equivalent to 66 g. nitrogen and calories to the extent of 6,550 is surely almost exceeding the physical capacity of the patient.

The plasma proteins, in particular the albumin, tend to fall and, if infection is present, the globulin may rise. Taylor *et al.* (1943a) have reported that of 81 burned patients studied, 40 had hypoproteinæmia, the degree of which appeared to be related to the severity of the burns.

In the later periods of the repair process and during convalescence there is a positive nitrogen retention, and the bodily losses are then made good. Restitution of the architecture of bodily function takes time.

Conclusion

In the foregoing paragraphs there have been sketched some of the main features of protein metabolism, which in itself is but one episode in cellular activity; not only is there change of material but there is change of energy. Although a little is known of the great activity of the organ proteins and of their continuous regeneration from the amino-acids of the fluids that bathe them, practically nothing is known about the regulation of this continuous chemical activity, which is so adjusted that there is normally no final quantitative or qualitative change in the composition of the tissues other than those appropriate to the stage of growth and sex of the organism. Here lies a practically limitless field of discovery.

In the past, pathology has been largely a study of morbid

anatomy and protein has been considered only in so far as it was the main material which took up dyes and so provided a picture of the histological structure. The pathologist is now tending to become a morbid physiologist and protein is no longer for him solely a clue to cellular configuration but is now an integral part of function, and his study concerns the regulation of those chemical processes which lead to deformation and dysfunction. A somewhat similar transformation is taking place in the field of medicine and surgery. Evidence of this is particularly to be seen in the new approach to the study of diseases of the liver and the reaction to trauma.

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AN EVALUATION OF THE VITAMIN-C POSITION

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Scurvy in human beings was one of the first diseases the ætiology of which was traced beyond any doubt to a faulty diet. It was also an interesting coincidence that vitamin C was the first among the organic accessory food factors to be isolated, identified and synthesized. Furthermore it was found that it could be synthesized with great ease and consequently at a lower cost than any of the other vitamins. This latter feature combined with certain chemical characteristics possessed by *l*-ascorbic acid has attracted a great number of uncritical workers, both in the clinic and in the laboratory, who were susceptible to the blandishments of a therapeutic novelty. It is not, therefore, surprising that the greater part of the literature on the subject is rather more plausible than convincing. Unfortunately, in spite of the strongly pressed advice of the cautious, this literature has had its influence in some fields where the authorities take charge of the public health. It will be seen from this paper that when the work done on the subject during the past ten years or so is brought into focus, an aspect is obtained which is quite different from that presented by the casual perusal of the voluminous and troublesome literature.

The Chemical "Methods"

One of the factors which contributed to the prevailing distortion of our knowledge of vitamin C was the application of the so-called chemical methods employed in its assessment. Pioneer biochemical researches have shown that antiscorbutic solutions were endowed with a strong reducing capacity which was more or less parallel with their antiscorbutic activity as assessed biologically on guinea-pigs. The most specific reagents in this respect were found to be dyes of the phenolindophenol class which became decolorized in the process of reduction. As was to be expected, vitamin C when isolated was found to reduce indophenol as well as other reagents. By using standardized solutions such as indophenol, iodine, etc., pure *l*-ascorbic acid can therefore be determined titrimetrically with the accuracy of a fraction of a milligram. This fact led to the development of multitudinous "methods" with "refinements," the purpose of which was not immediately evident to everyone. Unfortunately even indophenol is not specific for *l*-ascorbic acid and therefore, although a useful reagent in the hands of the cautious, its promiscuous use on extracts of animal and plant tissues led to the promulgation of faulty conclusions. Much misleading information was disseminated, especially when these "methods" were employed in the assessment of vitamin C in natural foodstuffs, particularly when cooked or processed. In heating products containing carbohydrates, certain antiscorbutically-inactive substances capable of reducing indophenol may be formed. When the heating is carried out in alkaline medium, reductone is produced from hexoses, whilst in acid solution reductic acid is formed from pectins and pentoses. The chemistry of the degradation process of the above precursors is complicated and at present obscure, but it was sufficiently known that it may seriously interfere with the assessment of vitamin C in such circumstances. For instance, while the indophenol reducing substance reductone may be formed in the process of heating in alkaline solutions, ascorbic acid is being simultaneously destroyed in the presence of air. The resulting indophenol titration can thus be entirely misleading as an index of ascorbic acid. It is therefore not surprising to find that according to some workers no destruction of vitamin C takes place—a synthesis is even sometimes indicated—when vegetables and fruits are heated in the presence of alkali. Recent work has shown that the indophenol titration may to some extent be corrected for non-active substances. The procedure nevertheless still remains a dangerous weapon in the hands of the uncritical.

The Phenomenon of "Saturation"

There seems little doubt that the chief reason for the exaggerated views held by many on the requirements of vitamin C for maintenance of good health is the confusion of thought that prevails concerning the phenomenon of "saturation".

One definition of "saturation" in man is the condition attained when a more or less constant daily amount of the vitamin C is eliminated in the urine as a result of ingesting a constant daily quantity of the vitamin above a certain minimum. Under normal conditions, especially in towns, deliberate attention has to be paid to the constitution of the diet in order to maintain a condition of "saturation". "Saturation" is therefore in this sense abnormal. Many people apparently in the best of health are known to be "unsaturated" or even not to eliminate ascorbic acid in the urine for long periods, especially in the winter months. The degree of "unsaturation" in such cases may differ within certain limits, but there is every reason to believe that the condition in the most "unsaturated" of this category could be induced in "saturated" people by their existence on a totally scorbutic diet within a period which is extremely short in comparison with the time necessary to produce frank scurvy. Yet such expressions as "hypovitaminosis C," "vitamin C subnutrition" and even "latent potential scurvy" and "subacute scurvy" are being recklessly applied to this condition by many writers and thus unwarrantably confusing it with the condition of "unsaturation" prevailing at the latent border-line of scurvy. At present there is no definite evidence to show at what stage "unsaturation" may really begin to have an unfavourable influence on health, but what evidence there is suggests that it is likely to be in the zone immediately preceding the frank manifestation of the disease. The literature contains few cases of experimental scurvy in human beings, but recently two such cases have been reported* which have every appearance of being well-controlled experiments, In one case no subjective or objective signs of scurvy were observed in the subject after 100 days on a scorbutic diet. In the second case a feeling of fatigue developed only at the beginning of the third month. Measurements of this weakness by an ergograph, however, showed that "the number of contractions at the rate of one per second made by the right hand against a standard resistance showed only a slight drop during the period of vitamin C deficiency". Further "there was almost complete freedom from respiratory infection throughout the experimental period, covering

* [Crandon, J. H., Lund, C. C. & Dill, D. B. (1940) *New Engl. J. Med.* **223,** 353; Rietschel, H. & Mensching, J. (1939) *Klin. Wschr.* **18,** 273.]

the months of October to May. Only two very transient and mild attacks of coryza were noted, each lasting but a few days. In previous winters it has not been uncommon for the subject to suffer from frequent, severe upper respiratory infections." It is important to note that in the first case also the subject recovered easily from a cold which he developed after 45 days on the scorbutic diet.

The abuse of the " saturation " phenomenon is actually worse than is outlined above. A number of workers, in measuring what they call the level of vitamin C nutrition, use " test doses " for the purpose. The procedure consists of administering massive daily doses of several hundred milligrams of ascorbic acid, the size of the dose used varying with the individual worker, until the patient eliminates ascorbic acid in the urine. There again, the criterion for the end-point varies with the individual worker. It may be anything from the first appearance of ascorbic acid in the urine, to the excretion of 50% of the " test dose " ! It is evident that in most of these cases the result is not only saturation but also the loading of the blood stream with large quantities of ascorbic acid. In measuring the " level of vitamin C nutrition " these workers are therefore adopting arbitrary levels imposed by themselves in accordance with their degree of enthusiasm. These " levels " unfortunately have no obvious connection with the ætiology of scurvy. In addition they are using a criterion which is by no means standard. It may therefore be fairly questioned whether the " saturation " tests justify the interpretation placed upon them. There is even no evidence that the vitamin C in the tissues of " saturated " persons is stored for reserve purposes. Results obtained with guinea-pigs point to the contrary.

The Plasma Ascorbic Acid

The concentration of ascorbic acid in the blood is influenced by the quantity of vitamin C consumed, by the ascorbic acid content of the tissues, and by the function of the kidney to eliminate the vitamin. In " unsaturated " subjects it is carried to the tissue and disappears from the blood stream within a short time. When the dose is small, little, if any, is eliminated by the kidney under these conditions. When the dose is very high, particularly when it is introduced parenterally, some may be voided in the urine even before " saturation " is reached. When " saturation " is actually attained there is a tendency for the kidney to eliminate the vitamin which is circulating in the blood, and if no further ascorbic acid is supplied to the system extraneously, the tissue ascorbic acid will evidently pass eventually into the blood and be eliminated. During the phase of " saturation " the ascorbic acid concentration of the blood is controlled by several factors, such as the quantity of ascorbic acid ingested, the time which elapses after ingestion, etc. When " unsaturation " sets in, owing to the consumption of small quantities, or to total abstention from ascorbic acid, the plasma content gradually sinks to very low values or zero. This need not necessarily indicate a vitamin-C deficiency, any more than the early stages of " unsaturation " as manifested by lack of urinary elimination of ascorbic acid. In the experimental case of scurvy mentioned above, the plasma ascorbic acid was determined daily and it was found that the content fell rapidly and reached the low value of 0·1 mg. per 100 ml. of plasma after about 9 days on the scorbutic diet. For about another 8 days the values vacillated at about that level and eventually the content uniformly declined to zero 41 days after the beginning of the experiment. It is doubtful whether ascorbic acid values of the order of 0·1 mg. per 100 ml. of plasma can be considered as significant. Even so, the experiment showed that the absence of ascorbic acid from the blood was attained after about 41 days on a scorbutic diet when normal health was enjoyed. It must be noted that in this subject the plasma ascorbic acid was zero for thirteen weeks before the first evidence of clinical scurvy became manifest. Therefore, like the " saturation " test, it cannot be accepted as a test for vitamin-C deficiency. Of course, in cases of latent scurvy which are indicated by a response to specific therapy, the zero value of the blood offers additional support to the diagnosis.

Vitamin-C Requirements

It is customary to measure the requirement of a dietetic constituent by the daily dose necessary to maintain good health. Such an assessment is fairly simple in the case of an animal. In the case of man, however, it offers, for obvious reasons, great difficulties, and consequently the minimum dose has to be deduced by indirect means. During the last three decades or so, much detailed experimental work has been carried out on scurvy in the guinea-pig, and indications have been obtained that a quantitative relationship exists between the guinea-pig and man in several respects.* The minimum daily dose of vitamin C for man can therefore be deduced with a fair degree of probability in the following way. A daily dose of about 2 mg. of ascorbic acid not only prevents the production of macroscopic and microscopic lesions of scurvy in guinea-pigs on a scorbutic diet, but apparently also enables the growing animal to lead a normal existence. About ten times this dose is required to attain the maximum concentration of the vitamin in the tissues, *i.e.* to attain " saturation." The dose necessary to maintain " saturation " in man as measured by the urinary output of ascorbic acid varies within certain limits with the individual and is of the order of 50 mg. per diem. If the ratio between the minimum " saturation " dose and the minimum protective dose is the same in man as in the guinea-pig, the minimum human requirements would be of the order of 5 mg. daily. Compared with the high targets now often recommended this figure may at first sight appear alarmingly low. When, however, the very low vitamin C content of the diets consumed by a great number of apparently healthy people for long periods in this country, particularly in the winter, is considered in relation to the very low incidence of scurvy, a minimum protective dose of 5 mg. does not appear to be surprisingly small. In view of these considerations the daily intake of 30 mg. recommended by the League of Nations Health Organisation Technical Commission on Nutrition seems to be a reasonable target, as it is in all probability several times higher than the minimum protective dose and would not require the consumption of excessive bulks of vitamin-C-containing foods even in the winter. It must, however, be observed that the above intake is intended as a target value and consequently does not require strict adherence. There seem to be many indications that only when this daily intake falls to a value below 15 mg., or perhaps even 10 mg.—in other words when approaching the minimum requirement—that precautions are required.

As scurvy is a disease which develops because of the absence of a dietetic factor, it follows that the introduction of the daily minimum dose of vitamin C into the food should be sufficient to cure the disease eventually. This is supported by the fact that improvements in patients suffering from scurvy have been observed on ordinary hospital diets poor in the vitamin even before treatment was commenced. In order to effect a rapid cure, however, large quantities of vitamin C may have to be administered at first. In deciding on the size of the dose the condition of the patient must evidently be taken into consideration, and therefore the form of administration and the quantity to be administered must be left to the discretion of the physician. Doses of several hundred milligrams of ascorbic acid have been successfully used in the early part of the treatment.

Vitamin C and Diseases other than Scurvy

The isolation and synthesis of *l*-ascorbic acid was followed by an indiscriminate search for the possible use of the compound in a number of diseases. In some diseases, like hæmorrhagic states different in their pathology from scurvy, and in Addison's disease, in which the response could have been expected to be of a definite nature, it was found to be ineffective. Claims concerning the merits of the new compound were made by some workers in respect of other applications, *e.g.* dental disease, skin conditions, cataract, gastro-intestinal disturbances, immunity and resistance to infection, wound healing and surgical conditions, pneumonia, tuberculosis and diphtheria. At the same time, however, these claims were contradicted by the evidence produced by other workers which militated against the suggestion that *l*-ascorbic acid was beneficial in the treatment of these diseases. Furthermore, when positive results were obtained, the position was often obscured by the failure of the workers to appreciate two cardinal facts. In the first place, in some cases such as wound healing, the improvement could often be traced to the rectification of a genuine pre-scorbutic

* [The author has discussed this relationship in more detail in the following papers: Kellie, A. E. & Zilva, S. S. (1939) *Biochem. J.* **33**, 153; Zilva, S. S. (1941) *Biochem. J.* **35**, 1240.]

condition. In other cases such as gastric and duodenal ulcers, the effect was confused with the cause. The undoubted presence in some instances of a genuine vitamin-C deficiency was evidently brought about by the insufficiency of the vitamin in the diet used in the treatment, and consequently the deficiency was not connected with the ætiology of the disease. In general it may be said that so far no convincing evidence has been produced that vitamin C has an influence on any condition other than scurvy.

Conclusions

A careful examination of the recent literature on vitamin C does not then appear to give quite the same impression as that obtained by the casual perusal of numerous contributions on the subject, which are more often than not characterized by a certain missionary spirit, and which are not always sustained by objective reasoning. The common misconception of the phenomenon of "saturation", with the consequent formulation of the high requirements of vitamin C for the maintenance of good health, has been followed by many spurious activities. It has encouraged a great deal of unnecessary experimental work in order to preserve the vitamin C in stored, cooked, processed and dried foods, etc.—by no means an easy problem—which, considering the abundance of vitamin C in the vegetable and fruit products of most countries, served no real purpose. The unjustifiable stress placed by the teaching of public bodies on the importance of ensuring high intakes of vitamin C must no doubt have been responsible for causing unnecessary strain on the public in general and on the housewife in particular, especially as the information disseminated was not always scientifically correct. It is indeed questionable whether the scientific knowledge on vitamin C obtained in the interval between the two wars has really added anything to our knowledge which has a bearing on practical nutrition. The crude assessments of the vitamin-C content of foodstuffs were in the early days in many ways more reliable than the supposedly accurate modern chemical "methods," as they were all carried out by the only dependable test, namely the biological test. If the accuracy of this assessment is not high, it is, at all events, sufficiently accurate for the classification of antiscorbutic foods such as is needed for practical purposes. It is true that synthetic *l*-ascorbic acid has its advantages in certain cases of human disease, but this is by no means balanced by the gross and unwarranted abuse of the compound for general nutritional purposes. No doubt this biochemically interesting compound calls for much research, and may even reveal some unexpected therapeutic merit other than as a specific for scurvy, in medical practice, but useful results in this field can be obtained only by laboratory workers and clinicians who can impose upon themselves the strictest rules of observation and control.

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OBSERVATIONS ON WATER METABOLISM

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All the chemical reactions, which together constitute life, as we know it, take place in water or at a water interface. Life without water, therefore, is an impossibility, and it is a well known fact that the body of an adult contains some 72% of water. It is not so generally known that the body at birth contains considerably more, but this is well established, and a fall in the percentage of water is one of the chemical characteristics of growth.

Effect of Reduced Intake

Man is surprisingly sensitive to a reduction in his water intake. Anyone can demonstrate this to himself and his friends by a very simple and perfectly safe experiment. All he (or she) has to do is abstain from all liquid for three or four days, and to eat only "dry" food such as biscuits, cheese, jam, sugar and chocolate. By the third day the experimenter will probably have lost from 8 to 16 pounds [about 3·5 to 7 kg.] of weight. He will be very thirsty, but not perhaps inordinately so, and he may even maintain that he feels quite well, though he can no longer eat the dry food, and finds it a little difficult to talk for long at a time. His friends, however, will have observed a gradual change in his appearance. His cheeks will have become paler than they ought to be, and somewhat sunken; his lips will look dry, and they will probably have developed a slightly bluish tint. If the subject of this experiment decides to bring it to an end, and drinks two or three cups of tea, his friends will probably notice the most dramatic change in his appearance, and within 10 minutes he will have recovered all his characteristic colouring and be looking the picture of health once more. It may, however, take him some hours of steady drinking to regain his loss of weight (Nadal, Pedersen & Maddock, 1941; Black, McCance & Young, 1944).

Sources of Water

Man gets the water for his daily needs from three main sources. The first and most obvious of these is the water, coffee, tea, beer, soup or other liquid which he drinks. The second is the water in the so-called solid food. This may be a considerable amount, for some foods which look very solid contain a surprising quantity of water. Cabbages and marrows, for instance, may contain 95% of water, and apples as much as 90%. Meat again is more, and bread not much less, than half water. The third source is the water formed during the combustion of the food materials, for all of them are normally burned to CO_2 and water. A gram of protein or sugar burns to about 0·5 cm.^{3} of water and a gram of fat to 1 cm.^{3}, so that the metabolism of 100 g. protein, 100 g. of fat and 400 g. of carbohydrate will give rise to about 300 cm.^{3} of water. This is not enough for a man's daily needs, but some insects such as clothes moths must be able to live on the water which they derive from the oxidation of their dry, if somewhat valuable, rations.

Water and Salt Balance

If, as already implied, man is to remain efficient, he must be maintained in water balance, and he must, therefore, be supplied with enough water to replace the large amounts which he loses every day through the lungs, kidneys, and skin (Peters, 1935). The air expired by the lungs must always be saturated with water vapour, and the daily loss through this channel comes to about 400 cm.^{3} in a person taking a minimum amount of exercise, and in a temperate climate the losses by the skin can be cut down to a figure not much greater (Futcher, Consolazio & Pace, 1944). If 1,000 cm.^{3} per day be taken as the amount of water required in temperate weather by the skin and lungs, no great error will have been made, but in a hot climate a man may lose 14,000 cm.^{3} by the skin in the course of a day (Hunt, 1912) and all this loss is essential if the man is to survive, for were he to stop sweating his temperature would rapidly rise to a level that was incompatible with life.

Now sweat is essentially a dilute solution of sodium chloride, but since it contains very much less of this salt than the plasma, sweating will always lead to a concentration of the body fluids. If, however, sweating is very profuse and continuous, considerable amounts of salt may be lost as well as water. If the subject now drinks water freely, he may so dilute his body fluids that he is seized with the most severe cramps and becomes seriously ill. This can be prevented by the simple expedient of adding a small quantity of salt to the drinking water of men who are working in a very hot atmosphere. It is wiser to do this than to rely on a larger intake of salt at meal times, for men may eat less food for many reasons and still continue working and drinking. Such men are very liable to suffer manifestations of salt deficiency. Salt, however, should not be added to the water unless the heat is very great and the subjects have free access to water while they are at work. It would be a great mistake to add salt to the drinking water if the supplies were limited.

Man loses a good deal more water through the skin than certain other animals. This gives him an advantage over

the dog, for instance, in a very hot desert climate, for he can do hard work and still keep cool by sweating. The dog does not sweat, and normally cools himself by panting and protrusion of his moist tongue, but in the heat of the desert sun the animal rapidly becomes a casualty because his skin becomes unbearably hot and his temperature steadily rises. In a more temperate climate, however, the dog would have the advantage over the man if both were deprived of food and water, for the man would soon get very thirsty, while the dog could live quite comfortably on the water in his own tissues, which were being broken down, and on his own water of metabolism.

The human kidney is forced to pass out about 500 cm.^{3} of water a day with the urea and other solids which it excretes. The actual amount of this obligatory excretion depends upon the amount of solids, and particularly the amount of salt, which the urine contains. If the diet has contained little salt, or if sweating has been profuse in a hot climate, the urinary chlorides may be low and the obligatory volume not much more than 300 cm.^{3}. It can be raised experimentally by taking salt (McCance & Young, 1944). The rest of the water passed by the kidney may be regarded as the surplus of intake over the essential requirements. This surplus amounts only to some 200 to 2000 cm.^{3} per day whatever the work or climate, which shows how well the intake is normally regulated by the desire to drink (Smith, 1937). This mechanism, however, is not perfect, for it is clear from the way in which people lose weight at the onset of really hot weather, and from the very small volume of urine passed during such weather, that the sensation of thirst may not be strong enough to prevent men getting into a state of hydropænia. It is, therefore, very important that administrators, medical men and others responsible for the welfare and efficiency of troops working in high temperatures should ensure that the men in their charge have free access to cool water day and night, and that they are encouraged to drink it. It is equally important to see that such working men are provided with enough salt to replace the amounts lost in the sweat.

Within the last few years the exigencies of war have forced men and women to live for many days and even weeks in open boats, and the question of drinking sea water has come up again and again. Now sea water may be regarded as a 3% solution of common salt, and the fluids bathing the cells of the body as a 1% solution. The kidney can excrete a 2% solution of salt, but no more, so that taking sea water by mouth can only lead to a concentration of the body fluids, which is the one thing that shipwrecked mariners are trying to avoid. But for the timely aids of science, which are now being made available (Spealman, 1944), Coleridge's lines are as true to-day as they were when they were written:

> Water, water, everywhere,
> Ne any drop to drink.

Marine Animals

How, then some may ask, do the marine mammals manage to survive, and how do the sea fish manage their water economy? The body fluids of these animals are no more concentrated than a man's, in spite of their hypertonic surroundings. The seals and whales need less water than man for they have no losses through the skin. They do not drink the water in which they live, in fact, they are careful not to do so, and only swallow their food after separating it from the sea water. They live on the water which their prey contains, and the water derived from its metabolism. The bony fish do drink sea water, but they have cells in their gills which excrete salt outwards, and so, although their kidneys are much less effective than those of mammals at excreting salt, they manage to maintain their body fluids at an osmotic pressure near to a 1% solution of sodium chloride. The elasmobranch fish have a different mechanism. Their body fluids contain 2% or more of urea. Their gills are impermeable both to urea and salt, and they have so much urea inside them that their body fluids have a higher osmotic pressure than the surrounding sea. Consequently water passes into the animals and provides their kidneys with the means of excreting the waste products of the body (McCance, 1936; Krogh, 1939).

Water and Salt in Illness

Unlike other animals, man can exist for a long time outside his natural habitat, but only if by his forethought he has provided himself with the things which are essential for life. Of these water is second only to oxygen, and it should always be given precedence over food in lifeboats and rafts when space is limited.

When a man is ill, his requirements for water are almost as great as they are when he is up and about, and they may be much greater, for some diseases are characterized by a forced excretion of water. If this excretion is taking place by the bowel, it will be accompanied by a loss of salt, and both will have to be replaced. The usual way of doing this is by giving isotonic saline intravenously, and this is often a life-saving measure, but the use of saline can be overdone—particularly in young children. The mistake is made by those who cannot distinguish between water requirements and salt requirements. Although the metabolism of salt is intimately associated with the metabolism of water, the requirements of the two should not be confused. Saline should be administered only if there has been a loss of body fluids, and preferably when this loss has taken place to the exterior by vomiting or diarrhœa. Internal "loss," as in shock, is a separate question, and it should be treated usually by the administration of human plasma. Patients in need of water and unable to take fluids by mouth, as may often be the case after surgical operations, should be given a 5% solution of glucose intravenously. This provides them with a certain amount of food, and water to make good their losses by the lungs and skin. The simplest way of making sure that a patient is being given enough water is to test the specific gravity of the urine (Lashmet & Newburgh, 1932; Coller & Maddock, 1935; Jones & Morgan, 1938; Arnott & Young, 1942).

The dangers of giving moderate amounts of saline with the idea of treating dehydration may be illustrated by the following simple calculation. Let us suppose that a patient is unable to take anything by mouth, and that, in consequence, he is given, during 24 hours, 1500 cm.^{3} of normal saline intravenously, *i.e.* 1500 cm.^{3} of water and 15 g. of salt. He will require 1000 cm.^{3} of this water to make good the unavoidable losses from his lungs and skin, and so his kidneys will have the impossible task of excreting 15 g. of salt in 500 cm.^{3} of water. The kidneys may excrete most of the 15 g. of salt, but they can do so only by drawing upon water which was already in the body. Hence the administration of 1500 cm.^{3} of saline could only make the patient more dehydrated, while 1500 cm.^{3} of 5% glucose would have met all his water requirements. The casual administration of saline to very young infants is even more dangerous than it is in adult life, for the infant kidney is much less capable of dealing with the superfluity of salt (Young, Hallum & McCance, 1941; McCance & Young, 1941). It is, in consequence, very easy to raise the concentration of salt in an infant's body fluids to a level which must interfere with the child's well-being (Aldridge, 1941). It is a safe rule never to give 1% saline except specifically to replace a loss by vomiting or diarrhœa. All other fluid given should be salt-free or contain much less salt, and 5% glucose or 5% glucose in 0·2% saline are satisfactory solutions for the intravenous route.

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MINERALS IN HUMAN NUTRITION

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Students of nutrition have never produced discoveries of such immediate popular interest as the sulphonamides or penicillin, but it is probably true to say that by their united efforts they have done more for mankind than all other medical investigators put together. Nor are their labours completed. Every year some new advance is made and more and more opportunities of applying existing knowledge are discovered.

Interest in mineral nutrition has always tended to centre on those elements which are likely to be deficient in quantity or which may do harm by their excess. Every physiologist knows that magnesium is essential for life and that zinc has recently been discovered to be a component of the carbonic anhydrase enzyme system, but nutritionally these two elements are uninteresting, for no one has ever produced evidence to show that either of them is likely to be deficient in human diets, or present in a dangerous excess.

Our knowledge of the laws which govern the absorption and excretion of minerals has expanded considerably during recent years, and some general principles have now become firmly established. One is that minerals are much more readily absorbed if they are in solution, and consequently any ion which precipitates another in the stomach or intestine will, *ipso facto*, prevent its absorption. Soluble aluminium salts, for example, will inevitably react with phosphates and precipitate them irreversibly in the gut (Street, 1942; Kirsner, 1943). This is the mechanism which enables phytic acid to impede the absorption of calcium, magnesium and iron, and probably other divalent metals such as zinc and lead (McCance & Widdowson, 1942; Wang, Liu, Chu, Yu, Chas & Hsu, 1942; Krebs & Mellanby, 1943). Oxalates prevent the absorption of calcium (Bonner, Hummel, Bates, Horton, Hunscher & Macy, 1938), and calcium of oxalic acid (Barrett, 1942), and those who fear the formation of oxalate calculi in their urinary tracts should therefore take plenty of milk with their rhubarb. The converse of this also appears to be true, and agents which improve the solubility of an element in the intestine also improve its absorption. It is for this reason that proteins improve the absorption of calcium and magnesium (McCance, Widdowson & Lehmann, 1942), for their constituent amino-acids form soluble coördination compounds with the carbonates and phosphates of these two metals. This all sounds so obvious when it is enunciated that it is essential to think oneself back into the state of knowledge existing only a few years ago to appreciate the work undertaken to establish it.

Calcium

The insolubility of the carbonate and phosphate of calcium at the *p*H of living tissues is the clue to much of the physiology of this element, for it explains its usefulness as a hardening agent in bones, teeth and shells, and also its poor absorption and tendency to precipitate in unwanted situations. The absorption of calcium from foods of different kinds has occupied a good deal of attention in recent years. This is very appropriate, for few foods except milk and cheese contain much of this mineral and there is not enough milk at present to supply the world with its calcium requirements. In times of calcium shortage the amount of calcium in the drinking water assumes greater importance, and it has been calculated (Widdowson & McCance, 1943) that up to 200 mg. of calcium per day may be obtained from drinking water in districts where the water is hard. This is as much as there was in the adult milk ration in Britain during the winter months of 1942 and 1943. The same authors have shown that the cooking of vegetables in hard water makes a small, but only a small, difference to the total calcium intake. Basu, Basak & De (1942), Basu, De & Basak (1942) and Basu & Ghosh (1943) have concluded that the calcium in the bones of small fish and in lime chewed with betel* nuts is partially absorbed, and Breiter, Mills, Rutherford, Armstrong & Outhouse (1942) have shown that the calcium in carrots is about half as well utilized as the calcium in milk.

There is no doubt that the calcium intakes of at least half the population of the world are much too low, and it is an unfortunate combination of circumstances that in these countries where the supply of milk is grossly deficient the diets very often consist mainly of whole cereals. As long ago as 1925 Mellanby discovered that there was some connection between the severity of the rickets developed by a growing dog and the type of cereal upon which it had been fed. Oatmeal was highly rachitogenic, whereas white wheat flour was relatively innocuous. At the time, and subsequently, the general importance of this discovery was missed, mainly perhaps because the experiments centred upon the production of rickets in growing animals and its cure by vitamin D. The last was therefore regarded as the important variable. In 1939, Harrison & Mellanby demonstrated that phytic acid played a large part in the rachitogenic action of cereals.

Until very recently it would have been safe to say that in spite of a great deal of highly suggestive work, vitamin D has remained the centre of interest in calcium metabolism, and the nutritional properties of cereals have been regarded as beyond practical control. The necessity for making the available wheat feed as many mouths as possible has revived interest in this problem, and McCance & Widdowson (1942), by means of prolonged metabolic studies with humans, showed that both calcium and magnesium were absorbed less freely from diets containing large amounts of brown flour than they were from similar diets containing white flour. Flour of 85 % extraction has also been shown to have a mild inhibitory action upon the absorption of calcium (Krebs & Mellanby, 1943). By adding sodium phytate to white flour, and by removing phytic acid enzymatically from brown flour, it has been shown (McCance & Widdowson, 1942) that the deleterious effect of brown bread on the absorption of calcium and magnesium in man is entirely due to phytic acid. The insolubility of the calcium salt of this complex acid radicle enables it to immobilize calcium from the rest of the diet in the intestine and so prevent its absorption.

Whatever the daily allowance of calcium ought to be, and it must, of course, differ from one individual to another, it is certain that many children in India (Wilson & Widdowson, 1942), Ceylon (Nicholls & Nimalasuriya, 1939), and Spain (Robinson, Janney & Grande Covián, 1942) have been existing on deplorably low intakes. Anything that can be done to raise the calcium intakes of poor people generally will undoubtedly benefit them, but the practical difficulties may be very great, particularly in a self-supporting, non-industrialized country such as India.

In Great Britain, flour was fortified with calcium in 1942, shortly after the extraction had been raised to 85 %. This measure was introduced partly to counteract the effect of the phytic acid in the 85 % flour, and partly to increase the calcium intake of a population whose milk supplies were reduced. Its wisdom has been demonstrated by the experiences of other countries which have not introduced such legislation. In South Africa, for example, where there has been a great increase in the consumption of high-extraction flours, measurable decalcification of the bones of the general population appears to have taken place (Meyer, Oosthuizen & Shapiro, 1942). Eire, however, has staged an even more significant large-scale experiment. Early in the war the shipping position forced the government of Eire to mill all the wheat available for human consumption to 100 %. The Dublin poor subsist largely on bread, and have very little milk, and this measure involved an enormous increase in their ingestion of phytic acid. No steps were taken to increase the amount of calcium in their diets, and in recent years there has been a serious increase in the incidence of rickets in the capital (Pringle, Reynolds & Jessop, 1943).

Striking evidence of the widespread improvement in mineral nutrition which has taken place in London during the last 25 years has been provided from comparative studies of the teeth of two large series of London schoolchildren by Mellanby & Coumoulos (1944). Teeth are laid down and formed long before they erupt, and it is necessary to seek the reasons for this improvement in earlier years. An increased intake of calcium has certainly played a part, but it is likely that vitamin D given to the children themselves, and to their mothers at the ante-natal clinics before they were born, has

* [The betel nut is the seed of the Areca palm (*Areca catechu*). It is usually chewed with lime and the leaf of *Piper Betle*, sometimes with the addition of other ingredients. The habit of betel chewing is extraordinarily widespread. It has been estimated that 200,000,000 of the human race are addicts.]

also helped to bring about the change. It is well known that vitamin D administered to women during lactation passes into the milk, but it has now been shown that administering calcium to mothers does not raise the concentration of this element in their milk unless vitamin D is given concurrently (Ritchie, 1942). It must not be overlooked, moreover, that there has been an improvement in the general nutrition of London children, and this may have been the most important factor of all in improving their teeth, for calcium metabolism is certainly connected with vitamin A and vitamin C, and the formation of bones and teeth must depend upon many other factors of which nothing is yet known.

Iron

It is well known to all analytical chemists that great precautions have to be taken to avoid contamination when iron analyses are being made, and metabolic experiments involving iron are difficult to carry out for this reason. Tables showing the amount of iron in foodstuffs are based on analyses of foods prepared with the greatest care to prevent contamination with extraneous metal. In the kitchen, however, no such precautions are taken. The housewife has her favourite kitchen knife, she has her mincer, and her chipped enamel pots and pans, and iron intakes as calculated from food tables should be regarded as minimum values. That actual intakes must sometimes be a great deal higher because of contamination from various cooking utensils was made clear from some experiments (Widdowson & McCance, 1943) in which it was shown that apples cut up with an ordinary kitchen knife and stewed in a chipped enamel pan contained 20 times as much iron as another sample of the same apples cut up with a stainless steel knife and cooked in a glass beaker.

Evidence has continued to accumulate during the past few years that the human body has no physiological method of regulating the excretion of iron, and that, in fact, very little is ever excreted. Thus, a patient with a severe hæmolytic anæmia, who was being kept alive by large and repeated transfusions, was found to be excreting none of the iron from her hæmolysed erythrocytes (McCance & Widdowson, 1943). The amount in the body must, therefore, be regulated by the amount absorbed, and Hahn, Bale, Ross, Balfour & Whipple, (1943) have published an account of an interesting though still very incomplete attempt to solve this problem by means of radio-active iron. They have found that the amount of iron absorbed by dogs depends upon the degree to which the mucosa has been saturated by the metal. Saturation by iron is rapid, and a large dose given a few hours before may block the absorption of a second dose containing the radio-element. The removal of blood does not at once desaturate the mucosa, but this follows gradually as the iron stores are depleted to make good the loss.

Fowler & Barer (1942) and Barer & Fowler (1943) obtained some rather unexpected results when they followed the rate of hæmoglobin regeneration in blood donors who were being treated with iron. They found, in confirmation of previous workers, that when blood was first withdrawn the administration of iron shortened the period required for complete regeneration, but they also found that this beneficial effect was not so obvious after the second removal of blood, and negligible after later withdrawals. The puzzling feature of those observations is that the donors who were treated must have been absorbing far more iron than the controls, and many times more iron than they can have required for the hæmoglobin synthesis. These stores of iron were full and their serum iron-content was high, and the results only serve to emphasize the incompleteness of our knowledge of iron metabolism. They should not, however, shake our faith in the use of iron salts as therapeutic agents or in the necessity for ensuring that infants, pregnant women, and indeed the whole population, receive enough of this element for their physiological needs.

Trace Elements

Zinc, copper and manganese are certainly necessary for human nutrition, but there is some doubt about cobalt. Sheep and cattle require this element, possibly in connection with the micro-flora which they harbour in their intestines. The main interest of lead continues to lie in its toxicity. The two trace elements which have been most in the nutritional limelight in recent years are iodine and fluorine. Most of the work on fluorine has centred round its capacity to induce mottled enamel, and, in optimum doses, to prevent caries without disfigurement (Irving, 1941; McClendon, Foster & Supplee, 1943; Deatherage, 1943). A considerable amount of goitre still appears to exist in rural England. Most of this is thought to be due to an iodine deficiency, and it has lately been recommended that table-salt should be iodized (*Medical Research Council*, 1944).

A curious fact about iodine metabolism has recently been investigated (Flexner, Bruger & Member, 1942; Bruger & Member, 1943). It appears that iodides are selectively concentrated and excreted by the salivary glands of man. It is difficult to see any biological value in this, for the iodide will only be reabsorbed again lower down the alimentary canal. These glands will not excrete the iodine in *tetra*iodophenolphthalein, although of course, the liver selectively does so.

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490

NUTRITIONAL FACTORS IN DENTAL CARIES AND PARODONTAL DISEASE

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The universal recognition of the high incidence of dental caries and parodontal disease makes it unnecessary to cite the exact figures disclosed by the innumerable clinical surveys made in many parts of the world. It is sufficient here to state that diseases of the teeth or of their supporting structures (gum and alveolar bone) in general affect more than 90 % of

mankind at some period of their lives. The fact that these mouth lesions seldom lead to direct fatality perhaps accounts, in some measure, for the relatively slow accumulation of knowledge required to cope with this grave public health problem. Their detrimental influence on general health is, however, gradually becoming more widely appreciated, and this awakening is reflected in the demand for more and more investigations designed to discover the ways and means of eliminating oral disease.

Before the last world war, the " hygienic " conception of dental pathology predominated. Both dental caries and so-called pyorrhœa were associated with dirtiness of the mouth and other factors were dismissed as of little account. Caries was said to be the result of decalcification of the tooth enamel by the acid products of fermenting carbohydrate food debris retained about the teeth. Pyorrhœa and other forms of gum disease were claimed to be caused by the putrefaction of mainly protein elements of the food, resulting in breakdown of the gum-epithelium and the formation of pockets along the roots of the teeth, allowing subsequent infection and rarefaction of the subjacent alveolar bone. In order to prevent such conditions, decreased consumption of white bread, sugar, sweets, etc., and a proportionate increased use of so-called " detergent " foodstuffs were advised, with vigorous brushing of the teeth and gums as an additional precaution. Even to this day, dental health propaganda is largely based on these purely theoretical conceptions—and caries and pyorrhœa remain rampant.

In 1918 M. Mellanby published the first of a series of reports on the influence of nutritional factors on the development and structure of the dental tissues and their susceptibility to disease. Apart altogether from the intrinsic physiological and pathological significance of Mellanby's findings, a new era of dental research was begun. For the first time the experimental method was applied to problems of dental health, and elaborate theoretical essays based on empirical impressions were replaced by verifiable facts. It is possible to describe here only a few of the more important results of this changed conception of dental science, but in general it may be stated that the most outstanding involve the realization that the resistance of the dental and parodontal tissues to disease is closely related to those nutritional factors which govern the health of the body as a whole.

Dental Caries

Miller (1890) showed that test-tube mixtures of saliva with bread, sugar and other high-carbohydrate foodstuffs fermented on incubation at body temperature. If an extracted tooth were immersed in such mixtures it became decalcified by the acid products of the media and assumed an appearance somewhat similar to dental caries *in vivo*. An analogous enamel decalcification was postulated as the initial phase of caries in the mouth, and from this idea sprang the carbohydrate-fermentation hypothesis of caries. Recently, however, the original experimental basis for the theory has been more critically examined. For example, the *in vitro* lesions in Miller's form of artificial caries differ considerably from those of caries *in vivo*. In the mouth the most susceptible areas of the tooth are its pits and fissures, but in " test-tube " caries produced by carbohydrate fermentation the first regions to be affected are the tips of the cusps and biting edge of the tooth—regions where the natural disease never begins (King, 1937); indeed, the pits and fissures of the tooth remain singularly unaffected by the artificial lesions until a large area of the rest of the enamel is diffusely decalcified. Moreover, it has been shown (Pincus, 1937, 1939, 1944) that disintegration of enamel can be induced *in vitro* by the action of certain proteolytic micro-organisms recovered from the mouth and having their optimal activity in *alkaline* surroundings; and in this instance the initial breakdown of enamel does take place in those situations where the natural carious process is known to arise. Not only are the pits and fissures of the tooth crown highly susceptible to the disease but there is also a much higher proportion of organic material in these areas. " These facts may be of no little significance if eventually it be found that the initial phase of caries is in the form of a lysis of the protein elements of enamel rather than a decalcification of its inorganic constituents " (*Lancet*, 1944). The findings may indeed provide a reasonable explanation of the now known association between the susceptibility of a tooth to decay and the structure of the tissues comprising it, which are in turn related to the calcifying properties of the diet. The clinical investigations of M. Mellanby and her colleagues (Dental Disease Committee, 1936; M. Mellanby, 1918, 1920, 1923, 1927, 1929, 1934; Mellanby & Pattison, 1926, 1928, 1932; Mellanby, Pattison & Proud, 1924) and others (Boyd & Drain, 1928; Boyd, Drain & Nelson, 1929; McBeath, 1932, 1934; Anderson, Williams, Halderson, Summerfeldt & Agnew, 1934; Schiotz, 1937; McBeath & Zucker, 1938; Boyd, 1940, 1943; King, 1940a; McBeath & Verlin, 1942) have shown clearly that nutritional factors such as vitamin D are of real significance in determining dental health, not only during the period of tooth development but even after calcification is complete. Further evidence in support of these contentions is supplied by an investigation in London reported during the present year (M. Mellanby & Coumoulos, 1944). On the other hand, no claim is made that increased consumption of foodstuffs rich in vitamin D, calcium and phosphorus will do more than reduce the ravages of caries, considerable as such a reduction might be.

Fluorine

Other factors undoubtedly play a part in the initiation and progress of the carious process and recent investigations have implicated the element fluorine. Numerous investigations have demonstrated that in communities whose drinking water contains about 1 part or more per million of fluorine, the incidence of dental caries is relatively low (Black, 1916; McKay, 1929; Ainsworth, 1933; Dean, 1938; Dean, Jay, Arnold, McClure & Elvove, 1939; Day, 1940; Sognnaes, 1940; Dean, Jay, Arnold & Elvove, 1941; Wilson, 1941; Murray & Wilson, 1942; Weaver, 1944). Indeed, the topical application of sodium fluoride to the teeth as a protective measure has already been reported (Bibby, 1942), while suggestions have also been put forward advising the reinforcement of the public water supply in areas where its original content of fluorine is below the 1 part per million level (McClure, 1943). That there are, however, still other factors to be discovered before full protection against caries can be accomplished is indicated by the findings of King (1944). A large field of research yet remains untilled before the human mouth can be rid of the dreaded fillings, forceps and artificial dentures.

Parodontal Disease

First it is well to mention that the term " parodontal disease " is here used to cover all departures from positive health of the tissues which surround and support the teeth, from very slight reddening and swelling of the gum margin to gingival atrophy, bone rarefaction and loosening of the teeth. Many aspects of the ætiology and pathology of the various forms of parodontal disease remain undiscovered. Both local and systemic agencies are involved but, while the nature of the former have long been recognized, identification of all the systemic factors is far from complete and their precise roles in the disease syndrome are still more obscure. Realization that food debris and tartar accumulating about the necks of the teeth lead to injury of the gum margin, and that these conditions are facilitated by irregular alignment or traumatic occlusion of the teeth, has led to the development of antiseptic and surgical measures designed to eliminate traumatic foci in the mouth (Stones, 1932a, 1932b, 1938). Such therapeutic procedures, aimed at establishing better mouth hygiene, have proved of real value in restricting the progress of established parodontal disease but unfortunately their effects are often only temporary. Moreover, while the severity of the lesions may undoubtedly be reduced, complete " normality " is seldom achieved. Indeed, without regular return visits to the dental surgeon for further observation and treatment, the original improvement may not be maintained. In other words, in the majority of cases there would appear to be some underlying systemic defect or defects which, if not determined and corrected, will retard if not prevent complete recovery of the affected tissues (King, 1943b). Foremost among these systemic disturbances must be placed deficient intake or utilization of various specific food factors, and account should be taken not only of acute vitamin deficiency but also of the possible influence of a sub-optimal supply of vitamins of relatively short or long duration.

Vitamin A

Since 1930 experimental investigations have demonstrated that vitamin A is essential for the proper development of the gingival epithelium (M. Mellanby, 1930; King, 1940b). Deficiency of this vitamin, especially during pre-natal and

early post-natal life, was observed to lead to epithelial hyperplasia and increased susceptibility to parodontal disease in later life. It was then found that, in the experimental animal, vitamin A deficiency also resulted in degeneration of the nerves to the jaws (M. Mellanby & King, 1934; King, Lewinsky & Stewart, 1938) and to excessive atypical alveolar bone, hypercementosis around the tooth roots, and retarded eruption with subsequent irregularity of the teeth (King, 1936; H. Mellanby, 1939). The end-result of all these changes was hypertrophy and later atrophy of the gingivæ, secondary invasion by micro-organisms, parodontal abscesses and loosening of the teeth—a series of events not unlike those associated with so-called pyorrhœa in man. The precise sequence of the changes in experimental A-deficiency is still not clear, but there is significant evidence that the nerve lesions in other parts of the body (and therefore presumably also in the mouth) are the result of compressive trauma from abnormal growth of the inner wall of the bony channels through which the nerves pass (E. Mellanby, 1938, 1941, 1943). The epithelial hyperplasia may be due to more or less direct stimulation of the epithelial cells, to loss of neurotrophic impulses from the degenerating nerve fibres supplying the tissue, to a combination of these effects, or to some as yet undetermined reaction to the lack of the vitamin in question. As regards the use of vitamin A in the prevention and treatment of human parodontal disease, little reliable information is available. On the basis of the animal investigations, however, we should be well advised to insist on a liberal supply of this food factor in the dietary of the expectant and lactating mother, and of her offspring at least up to puberty, in order to ensure that the gum epithelium and alveolar bone are properly formed. There would also seem to be an opening for vitamin A therapy as a supplementary measure before, during and after the surgical procedures employed in the treatment of established disease.

Nicotinic Acid

Since 1939 I have obtained some evidence * that the nicotinic acid component of the vitamin B_2 complex is of value in the treatment of ulcerative lesions of the gum (King, 1940c, 1943a; King & Francklyn, 1944). In this form of disease a fairly widespread superficial necrosis of many other parts of the buccal cavity may be seen, but the presence of one or more teeth appears to be an essential condition for its occurrence. The lesions bear no little resemblance to those seen in animals receiving diets deficient in the B_2 vitamins (Topping & Fraser, 1939). The precise ætiology of ulcerative gingivo-stomatitis (Vincent's disease) in man remains unsettled, but it is believed that the main pre-disposing agencies are local trauma of the gum, depressed general tissue resistance, often associated with and sometimes due to vitamin deficiency, and, coincident with or following these conditions, secondary non-specific bacterial action. During the past five years I have made detailed records of more than 800 cases of Vincent's disease and have personally treated some 500. In many instances administration of nicotinic acid or nicotinamide would seem to exert its beneficial influence by stimulating general tissue resistance, perhaps by restoring some broken link in the chain of tissue respiratory reactions, but supplementary local hygienic measures and sometimes tooth extraction are usually necessary to eliminate the traumatic foci and associated proliferation of anærobic micro-organisms. With regard to the possible infectivity of the ulcerative lesions much controversy exists, but my own opinion, supported by experimental self-induction of the characteristic disease (King, 1943), inclines to the view that the condition is likely to occur only in persons whose general resistance is depressed by defective nutrition or other illness, and whose mouths present more or less persistent traumatic foci.

Vitamin C

A large number of clinical investigations has been made of the effects of vitamin-C therapy on various forms of gingival disease. Many authors believe that the antiscorbutic vitamin is of value in supplementing local hygienic measures (Kramer, 1937; Roff & Glazebrook, 1940; Campbell & Cook, 1941; Kent, 1943; Stuhl, 1943). On the other hand, cases of typical scurvy have been described in which no gum lesions could be found (Blackstone, 1942); a similar finding was reported in the experimentally induced human disease (Crandon, Lund & Dill, 1940). Disappointing results have also been recorded in the treatment of both ulcerative and non-ulcerative gingivitis with a fairly wide range of ascorbic-acid dosage (King, 1944). An interesting suggestion regarding the possible benefit of vitamin C in the treatment of diseases of the gums is that it may assist in the healing of injuries caused by local traumatism (Cook, Davidson, Keay & McIntosh, 1944), but the evidence for this is not conclusive.

Other Nutritional Factors

No mention has yet been made of nutritional factors such as vitamin D, calcium and phosphorus, which are intimately connected with the development of bone and other calcified tissues. Obviously, deficiency of these food essentials, by causing rickets, osteomalacia and allied conditions, will lead to the production of defective alveolar bone and crowding of the teeth, a state of affairs which would be further accentuated by the excessive consumption of cereals having a high phytic-acid content. On the whole, however, most forms of parodontal disease show their initial lesions in the gum and not the bone and, for this and other reasons, further discussion of this aspect of nutrition is unnecessary here. In addition to those already referred to above, there are, however, a number of other vitamins which may play some part in the maintenance of parodontal health, more particularly other components of the vitamin B_2 complex; but the published reports concerning them are conflicting. One important point of agreement appears to be that in many animals the sum of the known vitamin B_2 components in their synthetic state is by no means as effective as the whole complex in the form of less artificial products, such as yeast and liver preparations. This, incidentally, is also in agreement with the experience of clinicians in parts of India, Malaya, Africa and elsewhere, in the treatment of some types of stomatitis not usually encountered in more temperate climates.

Non-Nutritional Factors

Brief reference should now be made to systemic factors, other than nutritional, which may have a bearing on susceptibility of the parodontal tissues to disease. So-called "pregnancy gingivitis" has often featured in the literature and, while its frequent association with nutritional defects should not be ignored, there is a certain amount of clinical and experimental evidence that sex-hormones may here be implicated (Ziskin, Blackberg & Slanetz, 1936; Ziskin, 1937, 1939). Many workers have also commented on the undoubted accentuation of gingival disease in subjects affected by diabetes mellitus (Goldstein, 1938; Cohen & Rudy, 1942; Rudy & Cohen, 1942; Lovestedt & Austin, 1943), but this field is still largely unexplored. Another aspect of the problem worthy of further study concerns the experimental demonstration of an association between herpetic viruses and ulcerative lesions of the gum in children (Dodd, Boddington & Johnson, 1939; Scott & Steigman, 1941). Finally, the nature and mode of formation of tartar or oral calculus is of much importance since its deposition is related to gingival disease, at least in the lower incisor and upper molar regions of the mouth. In this respect many interesting observations have already been made (Bulleid, 1925; Adamson, 1929a, 1929b; Smith, 1930; Wilkinson, 1935; Glock, Murray & Pincus, 1938), and further work may again reveal a connection between local and systemic factors. Indeed, as with dental caries, study of the interplay of local mouth conditions with changes in the organism as a whole would seem to offer the most promising line of approach to the whole problem of oral disease.

* [*see also BMB* **104, 530**]

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[5] [*see BMB* **526**]
[6] [*see BMB* **527**]

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DETERMINATION OF VITAMINS BY PHYSICAL, CHEMICAL AND MICROBIOLOGICAL METHODS

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Chemical, physical and microbiological estimations of vitamins are rapidly displacing the more tedious yet, as generally accredited, more reliable methods of biological assay. This is particularly true of that heterogeneous group of accessory food factors called the vitamin-B complex. The replacement of the time-consuming biological methods has originated from two developments in the vitamin field. First, the isolation and the characterization of the physical and chemical properties of vitamins paved the way for a number of colorimetric and physical methods. Then, the finding that many factors essential for animals are also neccessary for some micro-organisms led to estimation of the factors by their growth-promoting effects on the latter. Often, in fact, the characterization of a microbial growth-factor has preceded the establishment of its need by man.

However, in their present forms none of the methods of vitamin estimation have attained that accuracy which is desirable. The chemical methods, which depend upon the presence within the molecule of a non-specific chemical group, are limited in their accuracy, and vagaries in the nutritional requirements of micro-organisms impose a similar limitation on microbiological methods. However, it can be said that, in general, these methods give values for the vitamin content of foodstuffs and tissues which are not far removed from those given by the biological methods. For some time to come, the more specific animal tests will continue to be necessary as a check upon the less specific chemical and microbiological tests, but in evaluating these it should not be overlooked that there is a wide range of error inherent in animal tests. A blind faith in the infallibility of bio-assays may easily condemn methods which give the absolute, if not the physiologically available, vitamin content of a material.

War-time circumstances have had some part in the drive for the development of precise and rapid methods of vitamin determination. Experience of the last war has underlined the importance of vitamins in maintaining the nutritional status of the military and civilian population. Nor has the nutritional element been forgotten in the treatment of disease and of war casualties. In several ways analytical methods have helped to assure the vitamin intake of the individual. In checking the vitamin contents of natural and fortified foodstuffs, for estimation of the daily intake of vitamins, they have been used extensively. They have provided the means for numerous tests which, however inadequate they may be, seek to measure a person's nutritional status. No attempt will here be made to detail the methods in use or to exemplify the general applicability of any one method in the analysis of foodstuffs, tissues, blood and urine. For, often, the use of a method of proved value in the analysis of one foodstuff has not been fully explored in that of others.

General Considerations

It is particularly the water-soluble vitamins which have lent themselves to rapid estimation. Of these, aneurin and ascorbic acid, the first to be isolated, have received greatest attention; publications on the assay of vitamin C probably

exceed in number those on all the other vitamins. Of the fat-soluble factors, vitamin A can be estimated with certainty; for vitamin D there are promising methods and, for vitamins K and F, methods more in the developmental than finished stages are available.

Most of the vitamins are present in a combined form in tissues. Aneurin exists almost exclusively as a pyrophosphate in mammalian tissue (Ochoa & Peters, 1938). Nicotinamide is an integral part of the co-dehydrogenases, and riboflavin is a component of the flavoprotein enzymes. There is evidence for the combination of pyroxidin and pantothenic acid with protein. Vitamin A tends to exist in the esterified condition.

The existence of these combined forms necessitates, in most methods, liberation of the vitamin by chemical or enzymatic hydrolysis. In one or two instances the bound form can be estimated. For aneurin pyrophosphate Ochoa & Peters introduced a method, based upon the measurement of the CO_2 formed by the action of the pyrophosphate and carboxylase upon pyruvic acid, so delicate that amounts of 0.01 μg. could be determined. Estimations of this kind are important, as they determine the vitamin in a form in which it is biochemically active. Co-dehydrogenase 1 can be assessed by its growth-promoting effect upon *Hæmophilus influenzæ*.

No method for vitamin assay has yet reached perfection. Each one seems to have its advantages and weaknesses. The chemical methods lack the specificity of the biological, although they yield highly reproducible results. Methods which measure the intensity of fluorescence of a vitamin or its derivatives must take into consideration the phenomena of quenching; changes of fluorescence with *p*H; substances acting as light-filters; and the peculiarities of the chosen apparatus. It is clear that, for precision, fluorimetry demands close attention to experimental conditions. Measurements of the absorption-spectra of vitamins yield accurate results when careful standardization of the instrument and experimental conditions have been made and relatively pure substances are used.

In microbiological determinations, the great care needed in deciding the growth-medium is emphasized by the frequent modifications which are introduced to improve the method. For example, the specificity of an organism may be reduced by its growth-response to derivatives of vitamins; or the growth of the organism may be influenced by unsuspected toxins in the test solution. To avoid these difficulties it would appear most satisfactory to concentrate upon the use of a few selected organisms. Among these should be included *Lactobacillus casei*. Introduced by Snell & Strong (1939) for the microbiological assay of riboflavin, this organism has been shown, as a result of replacing peptone and yeast-extract by pure substances, to need a large number of factors and amino-acids for its growth. In fact, it is claimed that *Lactobacillus casei* may be used for the analysis of six B-vitamins: pyridoxine, riboflavin, biotin, pantothenic acid, "folic acid," and nicotinic acid (Landy & Dicken, 1942).

B-Vitamins

Aneurin: The first colorimetric estimation of aneurin was that of Kinnersley & Peters (1927, 1934), who found that in moderately alkaline conditions the vitamin gave a pink colour with diazotized sulphanilic acid, which could be stabilized by formaldehyde. A modification of this test was developed by Prebluda & McCollum (1936, 1939), who used diazotized *p*-aminoacetophenone. With improvements, this method has been used for the estimation of the aneurin content of national flours and bread (Platt & Glock, 1943). A hydrochloric-acid extract of the flour is treated with superfiltrol, upon which the vitamin is adsorbed. The washed adsorbate is treated with the diazo reagent, and the resulting dye is extracted with toluene. For flours and bread this procedure gave values in good agreement with those obtained by biological and fluorimetric methods (*Medical Research Council*, 1943). It remains to be seen whether it is applicable to other materials.

A popular method for aneurin analysis originated from the observation of Peters (1935) that oxidation caused the production of blue fluorescent substances from the vitamin. The product of the oxidation, called thiochrome, was isolated and synthesized by Todd, Bergel, Fraenkel-Conrat & Jacob (1936). In 1936 Jansen adapted the oxidative formation of thiochrome to the determination of aneurin. From that time his method has been subjected to numerous improvements and used widely in the analysis of foodstuffs and, in urine, in the so-called aneurin-saturation tests. Many materials must be treated initially with pepsin or takaphosphatase to free the aneurin from its combined forms. Purification of the extracts can be effected by adsorption of the vitamin upon *decalso*, a synthetic aluminium silicate, from which it is eluted by KCl solutions. Oxidation is achieved by alkaline ferricyanide and the thiochrome is extracted with *iso*butanol. The fluorescence-intensity of thiochrome can be estimated by matching visually against standards or by the Spekker fluorimeter. The method is not without its difficulties; one of which is the frequent occurrence of blue-fluorescent substance in tissues, another, the oxidative procedure. Its sensitivity is great. In the ingenious method of Hinton (1943), 0·05 μg. aneurin in cereal extracts can be determined with an error of ± 3%. The value of this method in those cases where only 1–50 mg. of material are available, is illustrated by Hinton's (1944) study of wheat grains in which 59% of the aneurin is localized in the scutellum.

As early as 1919, Williams suggested the use of yeast-growth as a means of assay of aneurin. Two microbiological methods may be considered sufficiently reliable to warrant mention: (*a*) the growth of *Phycomyces blakesleeanus* (Schopfer & Jung, 1937; Meiklejohn, 1937; Sinclair, 1938, 1939); (*b*) the fermentation of yeast (Atkin, Schultz & Frey, 1939). The growth of phycomyces is extremely sensitive to minute amounts of aneurin and, at present, it provides the only satisfactory means we have for gauging the level of aneurin in blood. To appreciate its limitations, the various papers of Sinclair (1938, 1939) should be consulted. Equally sensitive is the method of Atkin *et al.* (1939). It is based on the measurement of the rate of fermentation of sugar by yeast, using a Warburg or similar apparatus. With flours and bread it gives results as good as those obtained by other methods.

Riboflavin: This vitamin has a yellow-green colour and an intense yellow-green fluorescence. This property has been used in several methods for the determination of the vitamin. Yet fluorimetry does not appear to have gained the favour that investigators have bestowed upon the microbiological method. This is probably because no easy means have yet been found which, at the same time, effect a concentration of the vitamin and separate it from the substances interfering with its fluorescence. From the success which has attended the use of non-aqueous solvents and adsorbents in the isolation of riboflavin from urine (Najjar, 1941) and wheats, bread and milk (Barton-Wright & Booth, 1943), a more extensive use of the fluorimetric method is predictable.

At the moment, the microbiological method seems the more hopeful, and comparison of results with those obtained by biological methods, shows that they are almost as reliable. *Lactobacillus casei* is the organism ordinarily used. Under carefully standardized conditions, the growth of the organism is proportional to the concentration of riboflavin, and can be measured by titration of the amount of acid formed by the organism. Applied to the assessment of the daily intake of riboflavin of military personnel and workers, the method has shown that the daily consumption of riboflavin approaches 2 mg.

Nicotinic acid: Most chemical methods for the estimation of nicotinic acid are based upon König's reaction. The product of the rupture of the pyridine ring is condensed with a primary aromatic amine (aniline, *p*-aminoacetophenone, metol), to give a polymethine dye (Melnick & Field, 1940; Harris & Raymond, 1939; Kodicek, 1940; Bandier, 1939). The methods are considered specific for nicotinic acid; pyroxidin, another pyridine compound, does not give König's reaction. Natural pigments are a source of interference with the measurement of the coloured dye, and in plant-materials erroneous results arise from chromogens and pyridine-like substances. The method has permitted a much wider survey of the distribution of nicotinic acid in foodstuffs than the biological tests, for which the dog is the most suitable animal.

Application of the method to blood, where the greater part of the vitamin is in the erythrocytes, suggests that the level of nicotinic acid is not a good criterion of nutritional status (Carter & O'Brien, unpublished observations). In

fact, little difference has been found in the blood nicotinic-acid of normals and pellagrins. It is possible that a more suitable means of studying nicotinic-acid deficiency lies in the estimation of nicotinamide methochloride (Najjar & Wood, 1940; Huff & Perlzweig, 1943; Ellinger & Coulson, 1943). The elimination of this substance in the urine is dependent upon the amount of nicotinic acid in the diet, although it may also be related to the availability of methyl dontors in the body (Ellinger & Coulson, 1944). In general, the results of the chemical and microbiological methods are in agreement. *Lactobacillus casei* and *Lactobacillus arabinosus* are preferable to other organisms for the assay of free nicotinic acid. While improvements are still being made in the medium to enhance the response of the organisms to nicotinic acid, it is inadvisable at present to recommend any particular technique.

Pyroxidin: Pantothenic acid: There is no strong evidence pointing to an important rôle in human nutrition of either pyroxidin or pantothenic acid, although the detrimental effects of their absence from the diet of the rat are well established. Only a brief mention of some of the methods used in their determination will therefore be made. In virtue of its phenolic group, pyroxidin gives a blue colour with di-halogen quinone chlorimides, due to the formation of indophenols (Scudi, Koones & Keresztesy, 1940). On this reaction is based a method of estimation which has been applied to foodstuffs. Microbiologically, pyroxidin can be assayed by its growth-promoting effects upon *Lactobacillus casei*, *Saccharomyces cerevisiæ* (Williams, Eakin & McMahan, 1941), or the mould *Neurospora sitophila* (Stokes, Larsen, Woodward & Foster, 1943). Modifications have been necessary to obtain better agreement between these tests and the biological tests when they are applied to natural materials. It cannot be said that the tests have reached their final forms.

It is too early to judge the value of the methods of assay of pantothenic acid. Its identification as one of the growth-factors of yeast naturally suggested that its growth-stimulating power upon bacteria would provide a suitable means for its assay. For this purpose *Lactobacillus casei* has been employed for the measurement of the pantothenate content of tissues and foodstuffs. In blood, pantothenate would appear to exist in a free condition and also bound to protein, from which it must be freed by acid hydrolysis to be rendered available for microbiological estimation.

Ascorbic Acid

Plants can synthesize ascorbic acid with remarkable ease. So far no bacterium has been found which requires vitamin C for its growth. This vitamin can be estimated by several chemical methods, which draw upon the reduction of dyes. It was Zilva (1927) who first found that certain concentrates of the vitamin reduced phenolindophenol. But it is upon Tillmans' (1930) observation that the capacity of foodstuffs to reduce 2 : 6 dichlorphenolindophenol ran roughly parallel to their antiscorbutic potencies, that present methods are based. The method consists in titrating the vitamin-C extracts, usually made with metaphosphoric acid, with Tillmans' dye, and it has been used to determine urinary elimination of the vitamin and its level in plasma and leucocytes. Most of these determinations have as their object an assessment of the nutritional status of the individual as regards vitamin C. Although they have given valuable information, still further knowledge is required, not only for their proper interpretation, but also for their absolute evaluation.

Since the time of Tillmans' discovery, numerous modifications of the dichlorindophenol method have been made to render it more accurate in the ascorbic-acid assay of foodstuffs. The chief object of most of them is to circumvent the interference of other substances which reduce the dye. Among these compounds are iron and stannous salts, sulphides and thiosulphates. More important are biological compounds like cysteine and certain ene-diols, including reductones and reductic acid, which, in their chemical behaviour, are difficult to distinguish from ascorbic acid. These substances occur in caramelized and fermented products. Reductone can be produced by alkaline treatment of glucose and other sugars. The formation of this substance may not occur when foodstuffs are heated, but other substances of a "reductone" type may arise when sugar is heated to a *p*H of 5·4, and in dehydrated foodstuffs submitted to heat treatment.

For some time after Tillmans' introduction of 2 : 6 dichlorphenolindophenol, doubt of the reliability of dye methods lay buried beneath the abundant evidence of the good agreement between this chemical method and the animal tests upon a variety of foodstuffs. It is now clear that in many commercial products due allowance must be made for reductones and like substances. An interesting observation of Lugg (1942) of Australia suggests a possible means of differentiation between ascorbic acid and other indophenol-reducing substances by their different rates of reaction with formaldehyde at different *p*Hs. Mapson (1943) introduced a modification of the above procedure with the object of dealing particularly with the elimination of reductones. Lugg's principle has been under critical observation by Snow & Zilva (1943, 1944), who point out its limitations when applied to the estimation of ascorbic acid.

A note on the method of ascertaining the end of the oxidation of the dye-stuff with ascorbic acid is perhaps worth while. This may be achieved by usual observation of the end-point in the titration of dye with vitamin, or photometrically, or electro-chemically. The last has the advantage of permitting titration to be made with coloured fluids.

Another complication to be considered in estimating ascorbic acid lies in our ignorance of the amount of dehydro-ascorbic acid in tissues. In fresh fruits, vegetables, blood and urine, dehydroascorbic acid has been considered to be present in negligible quantities. It has usually been estimated as ascorbic acid in materials which have been gassed previously with hydrogen sulphide. This reductive procedure is not very attractive. It is tedious and creates false values for dehydroascorbic acid by the formation of additional reducing substances. Dehydroascorbic acid, however, yields an hydrazone with 2 : 4 dinitrophenylhydrazine. Roe & Kuether (1943) have applied this reaction to the determination of dehydroascorbic acid and ascorbic acid in blood and urine. Compared with other methods, Roe's procedure gives similar results for ascorbic acid. In fact, the method gives the same result as the titrimetric and the photometric, from which it would appear that pyruvic acid and glucose interfere only slightly (Higgins & O'Brien, unpublished observations). Blood and urine lack the complexity of plants and processed materials, in which reductones and other interfering substances are found. This may impose a limitation to a general application of Roe's method.

Vitamin A

In addition to biological assay, two methods, one physical and the other chemical, are in general use for the estimation of vitamin A. Introduced by Drummond & Morton (1929), the physical method has as its basis a property of vitamin A concentrates, namely a selective adsorption in the ultraviolet band with a maximum at 328 mμ. Its accuracy is well attested by many workers, but hardly compensates for the time which its intricate and expensive apparatus demands for an estimation. Accordingly a preference has developed for colorimetric and photometric methods. Pursuing the observations of Rosenheim & Drummond (1925), who showed that vitamin-A-rich oils gave colours with arsenic trichloride and trichloracetic acid, Carr & Price (1926) found that a blue compound was formed by such oils and antimony trichloride in chloroform. This colour, which is due to a light-absorption at 617–620 mμ and 580–585 mμ, could be matched with the standard glasses of the Lovibond Tintometer.

The Carr-Price reaction led to the development of several comparative methods. They are all open to errors, whose sources lie in differences between the light-absorption of (*a*) the colour standard or glasses and (*b*) the unknown; the difficulties of visual matching; non-linear relationship of the vitamin A concentration and readings, and so on. They have a further weakness in that they do not permit that speed of manipulation so necessary for dealing with the rapid fading of the blue colour.

The introduction of the photo-electric absorptiometer of the type designed by Evelyn, Yudkin and others, has removed several of the objections of the colorimetric methods. The

photo-electric absorptiometers have advantages in the speed at which measurements can be made, and in an objectivity which eliminates the personal factor. Their applicability can be extended by the choice of filters. By the use of filters isolating a narrow spectral band coincident with that of the maximum absorption at 620 mμ of the blue colour of vitamin A and antimony trichloride, or with that of the yellow colour of carotene, both substances can be estimated in the same oil or extract. The sensitivity of such instruments is such that determinations on 3 ml. of plasma are possible with an error of ±5 %.

With blood plasma, it would appear inadvisable to free the carotenoids by preliminary alkaline hydrolysis. This treatment causes low results, probably on account of destruction of the polyenes. Ether-extraction of the plasma after precipitation with alcohols ensures also complete removal of the carotenoids. The yellow petroleum ether extracts are then used for the determination. Even when great care is taken in the preparation of antimony trichloride reagent, and in rigid standardisation of the conditions of estimation, fading of the blue, with the small amounts of pigments in the extracts, constitutes a problem. Urban, Milder & Carruthers (1943) recommend that photo-electric measurements of blue colour of vitamin A and carotene with $SbCl_3$ should be made at 0°, a temperature at which the rate of fading is considerably reduced. From a study of the rate of fading, Hoch (1943) has been able to apply corrections to his values for vitamin-A concentration. By photographing the reaction-mixture with light of a selected wave-length range, he was able to measure the antimony-trichloride : vitamin-A colour within 2 seconds of the addition of the reagent to the vitamin. In this way it is possible to analyse the carotene and vitamin content of 0·1 ml. or less of serum—the amount obtained with a finger prick. This method may prove valuable in vitamin-A estimations upon the blood of infants or small animals.

Vitamin D

In the case of other fat-soluble vitamins, among which the vitamins D rank first in importance, no particular methods of determination have gained general approval. Bioassay still remains the most reliable means of determining the antirachitic potency of natural products. Nevertheless physicochemical technique combined with chromatography may resolve many of the obstacles to the development of a method more rapid than, but as satisfactory as, the biological. The amount of vitamin D is highly variable in oils and, with all of them, particularly the less potent, a preliminary step in most methods has been a concentration of the vitamin in a non-saponifiable fraction. With such fractions, spectrophotometric and colorimetric analyses are vitiated by the presence of sterols, such as cholesterol or 7-dehydrocholesterol, and of vitamin A. Of several colour reactions for vitamin D, the one with antimony trichloride is most promising. Especially is this the case when the reagent is prepared by the solution of anhydrous $SbCl_3$ in purified chloroform to which has been added acetyl chloride. As little as 2 μg. of vitamin D added to this mixture produces a yellow colour with a maximum absorption at 500 mμ.

The reaction has been used by Ewing, Kingsley, Brown & Emmet (1943) in a procedure of some interest. By chromatographic adsorption upon superfiltrol, the vitamin D and sterols are freed from vitamin A ; a second chromatographic adsorption separates vitamin D from the sterols. Determinations of the extinction coefficient, $E^{1\%}_{1\,cm.}$ at 500 mμ for the products of reaction of the mixture of sterols plus vitamin, and of the sterols alone, yield values from which the vitamin-D concentration can be computed by difference. Despite its lengthy procedure, the method gives results in agreement with those of biological assay. It does appear to represent real progress towards a method satisfactory for the analysis of oils and of natural and fortified milks.

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SOME PRINCIPLES OF WAR-TIME FOOD POLICY

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When Great Britain was involved in war in 1939, the first plans of the Ministry of Food at that time tacitly assumed that supplies of the foods then being eaten would continue and that, subject to the guarantee of a minimum share of those items which were scarce, the public could be left to choose its own diet. Very soon, however, it became apparent that some more rational food policy would have to be adopted and only a few weeks before the fall of France an intelligent plan was drawn up based on nutritional principles. The broad scientific basis of this plan has remained unshaken throughout the war. That is to say, the national diet, whether derived from home-grown items or from imported

commodities, has been based on the provision of food calculated to allow sufficient nutrients per head of the population for adequate health, with a free carbohydrate intake to supplement individual needs.

We now can draw up a table showing the average needs for each of ten or twelve separate nutrients for each different class of individual making up the population. Furthermore, it is possible to compile a table showing the nutrient composition of the foodstuffs which enter into the national diet. Thus, on the basis of these two lines of approach, an adequate food policy can be established. In considering this policy it is, perhaps, easiest to take each of several nutrient factors separately.

Calories

The energy value of a diet, expressed as calories, is in some respects the nutrient concept of most fundamental importance. To say that a man is not getting his calorie needs is to say that he is hungry. People can live for a very long time with too little to eat, but they will suffer. Muscular efficiency, and with it the capacity for industrial work, will be affected. The alertness and vigour of the population will decrease and there will be a loss of weight.

All foods provide energy for the body. The staple energy-foods are, however, cereals and potatoes, although, owing to the high proportion of water in potatoes, bread is, weight for weight, three times as good a source of calories. In the same rank as cereals comes sugar. Finally, more than twice as concentrated as any other food, fats are the richest of all sources of calories.

As the physiological need for calories is dependent on the amount of physical work a man does, and as the requirement immediately changes if an individual finds himself, if only for a short period, doing work of unaccustomed activity, the strict rationing and control of calories is difficult. The British policy has been, therefore, to provide for an unrestricted consumption of bread and potatoes. The Germans were forced to introduce a complicated rationing scheme for calories at an early stage in the war, principally because their nutritional position was less secure than that of the British. There is always an element of danger in a food plan when people do not have a cheap and unrestricted supply of an " energy " food.

Two periods in war-time food administration in the United Kingdom throw an interesting light on the national calorie consumption. Although cheap bread and potatoes have always been available in the shops, there is little doubt that, during the winter of 1940 and the early spring of 1941, people tended to eat food of insufficient calorie value and in those cases there was a loss of weight. This under-consumption of food was due to the fact that the diet was, although reasonably adequate nutritionally, uninteresting and lacking in variety. When more variety could be introduced, following the passage of the U.S.A. " Lease-Lend " enactment, the situation improved. The second period of strain on the calorie situation came about a year later. At this time, the shipping situation had become so serious that the possibility of not being able to continue to allow unrestricted bread consumption had to be faced, and a detailed plan for bread rationing was worked out. Fortunately the supply position improved and this fear was dispelled.

A number of expedients have been adopted during the course of the war to maintain the supply of calories. As animals can never produce the amount of food equal to the calorific value of the crops which might be grown on the same area, extensive tracts of country were ploughed up and wheat and other cereals which could be used in bread were grown on them. A large increase also occurred in the production of potatoes. Between 1938/39 and 1942/43, it was estimated that the increase in home-grown calories amounted to 30 %. Similar in principle to the transfer of land from the feeding of animals to the direct feeding of men, was the use for human food of a proportion of grain offals normally used as fodder. Before the war, 72 % of the wheat was extracted in the production of flour. Immediately the war began, the extraction was increased to 75 %. At the end of 1941, however, when Japan made her attack, it was decided to use 85 % of the grain for human food.

In parallel with the changes made to increase the production of calories at home, great efforts were made to import calories economically from abroad. By June, 1943, although the amount of foodstuffs imported had been reduced by 50 % the amount of calories imported was reduced by only 15 %. This result was largely achieved by a heavy reduction in the tonnage of animal feeding stuffs imported, and the choice of more concentrated items in place of bulky foods such as fruit. Meat was imported boneless or canned, and milk and eggs were brought in the dried state.

Fat

The amount of fat available in the diet has an important bearing on calorie consumption. Fat is the most concentrated of all sources of energy ; when there is a shortage of fat, it is inevitable, therefore, that the bulkiness of the day's food must increase. Dietary fat can be classified under two heads : " visible " fat can be said to comprise such things as butter, margarine and cooking fat ; " invisible " fat will include the cream in milk and the fat in bacon and meat. During war-time, fat is hard to get and it may be advisable to sacrifice " invisible " fat in order to maintain a reasonable supply of " visible " fat for cooking and other purposes. In Britain, butter, margarine and cooking fat are rationed, and it has been possible to keep the total ration steady. But, while it has not been found necessary to go to the length adopted by the Germans, who have to skim their milk in order to maintain supplies of butter, a certain amount of " invisible " fat, mostly from meat, has been used to increase the supplies of " visible " fat.

The total amount of fat, " visible " and " invisible," available per day per head of the population was calculated to be 131 g. before the war. At the end of 1940, the figure had fallen to 115 g. and, by the second quarter of 1941, it had reached 104 g. per head per day. This was one of the worst periods of the war with respect to food supplies. By the end of 1943, the figure for fat was about 111 g. It can thus be seen that the reduction in total fats in the diet amounted to 21 % in 1941 and that the more recent figures represent a 15 % reduction on the pre-war level. As one of the most important nutritional functions of fat is to provide calories in a compact form, it is very difficult to fix scientifically a minimum for the national diet, as a reduction in calories from fat could be made good by an increase in calories from, say, bread. The figures for fat consumption given above, in fact, represent more than 30 % of the diet's calories, which is a value sometimes thought to be physiologically desirable. It is, however, true that, when the amount of fat fell as low as it did in 1941, the diet became correspondingly unpalatable and bulky. This bulkiness may, under some circumstances, be crucial. For example, children who have high calorie needs and comparatively small stomachs may not be able to eat an adequate diet, even if it is put before them, if it is unduly bulky. For this reason, the allocation of fat permitted by the Ministry of Food for school meals has always been greater than that permitted for any other type of catering.

Protein

" If you look after the calories, protein will look after itself." This remark of Starling's is broadly true when the total protein in the diet is considered. It is true because, in war-time, almost every food contains a proportion of protein sufficient in the aggregate to give 1 g. of protein per kg. of body weight, which is usually accepted as a rough guide to an adult's needs.

A fraction of the total protein supply which is of especial physiological importance is that derived from animal foods. In the pre-war years, a steep gradation existed in the amounts of the most important sources of animal protein, milk, meat and even cheese, eaten by the rich and by the poor. The Ministry of Food imposed a uniform ration of meat, bacon and cheese for the whole adult population. Milk and eggs were also distributed as evenly as possible to adults. In order, however, to provide mothers and young children with an increased supply of animal protein, for which they have a specific physiological need, a priority allowance of milk was made under the National Milk Scheme for these groups. A prior claim on eggs was also established. An important aspect of milk distribution was a great expansion of the " Milk in Schools " scheme. These measures were aimed to distribute the available animal protein evenly over the population, except in the case of those individuals whose physiological needs demanded more.

By the beginning of 1941, however, national supplies of animal protein seemed almost inadequate for the national needs. When, therefore, the U.S. were able to offer a choice

of food under Lease-Lend, an urgent request was made for supplies of canned meat and fish, cheese, dried milk and dried eggs, all of which were intended to stabilise the situation in respect to animal protein.

Calcium

One of the most consistent deficiencies in the pre-war British diet was a shortage of calcium. Much of this shortage was due to disparities in the consumption of milk and, indeed, to the consumption over the country as a whole of too little milk. In the years immediately before the war attempts were being made to increase the total amount of milk drunk, and it was the deliberate policy of the Ministry of Food to continue these efforts even during the difficult war-time period. Since 1939 the total consumption of liquid milk has risen 28 %. Because of the organization of general distribution, and because of the priority claims of expectant and nursing mothers and of children, the consumption among some sections of the wealthier has fallen, but there can be no doubt that the operation of these plans has tended to improve the nutrition of the country as a whole. It has certainly effected a general improvement in the figures for calcium intake.

Subsidiary to the emphasis on milk as such, there has been a recognition by the Government of the nutritive importance of cheese and dried separated milk. These products not only provide good protein but are also sources of calcium and riboflavin. Every effort has been made to introduce the maximum proportion of these foods into the diet. The distribution of cheese is principally made by means of the standard ration equal for all. Part of the dried separated milk supply is directed to children, who have high calcium needs, by encouraging its use in the preparation of school meals. It is also distributed as *National Household* milk to hospitals and other institutions.

Perhaps the most important step to improve the calcium content of the diet has been its incorporation, as chalk, in bread. One of the disadvantages of extracting, in the process of milling, an increased proportion of the grain for human consumption, is that the amount of phytic acid present in the loaf is increased. Phytic acid is derived from the outer layers of cereal grains and possesses the unfortunate property of rendering a proportion of dietary calcium unavailable to the body. The addition of calcium to *National* flour was calculated to neutralize this effect and to improve calcium intakes. In April, 1942, the Ministry of Food imposed on millers the obligation to add 7 ounces [about 200 g.] of chalk (*creta preparata*) per sack of 280 pounds [about 130 kg.] of 85 % wheatmeal flour. This addition represents a supplement of about 180 mg. of calcium to the average daily intake.

The consumption of green vegetables, which has been so greatly encouraged during the war, also serves to increase the calcium intake.

Iron

The restriction in supplies of meat might have been expected to reduce still further the deficiency in iron among poorer people. The first step to prevent such an occurrence was the uniform rationing of available meat. Next, the change in the character of the bread caused an increase in supplies of dietary iron, and, finally, an increased consumption of green vegetables did the same. The introduction of 85 %-extraction flour made a substantial addition to the iron content of war-time diets. This *National Wheatmeal* contains about 10 mg. of iron per pound [about 0·454 kg.], whereas the pre-war type of white flour contained about 5 mg. per pound.

Vitamin A

Although a great deal of research has been done on vitamin A since its discovery in 1913, it is not possible to be dogmatic about the quantities required in the diet. It is known, for example, that the dietary allowance need not be so great if all the vitamin A is derived from animal sources and, on the contrary, if the vitamin activity is derived largely from vegetable foods the requirement will probably be more. In spite of this element of doubt as to quantitative needs which, for that matter, extends to other vitamins, it is likely that deficiency of vitamin A was widespread in pre-war British diets. Unfortunately, it has not proved possible entirely to rectify the situation. Several steps have been taken to provide vitamin A in the war-time diet. The vitamin has been incorporated in margarine to the extent of about 500 international units per ounce [about 28 g.]. The encouragement of the consumption of green vegetables has in part been proposed in order to increase the intake of vitamin A. Among the best vegetable sources of vitamin A is, however, the carrot, and the production of this vegetable has consistently been increased and its consumption encouraged. Another source of vitamin A to which special attention has been directed is dried egg, which has been a notable war-time development. All these foods are intended to maintain the diet of the adult population. Young children, however, cannot be expected to eat large amounts of green vegetables and carrots. For those below the age of 5 a special issue of cod-liver oil has been made available. This oil, put out by the Ministry of Food, has a minimum vitamin A content of 1000 international units per g. Although foodstuffs had previously been distributed to satisfy specific nutritional needs, and in this connection milk-issues and the provision of school meals to necessitous children will be remembered, the provision of a vitamin concentrate such as cod-liver oil to infants and young children is probably the first example of the distribution of a specific nutrient without distinction to all those who have a special need for it. Besides young children, pregnant and lactating women have been provided with a national issue of a vitamin-A concentrate.

Vitamin B_1

Early in the war there was a proposal to add synthetic vitamin B_1 to flour in order to overcome a bad deficiency in the pre-war diet of the poorer people. This plan was being put into effect when, early in 1942, the shipping situation became so threatening that it became necessary to raise the rate of extraction of flour to 85 %. The result of this change in milling practice was to increase markedly the vitamin B_1 of the national diet. Vitamin B_1 is concerned in the utilization of carbohydrate in the body. In peace time there are two important carbohydrate foods which do not contain within themselves sufficient of the vitamin for their own utilization and which thus combine to cause a net deficiency in the diet. These are white flour and sugar. Under war-time control, the amount of sugar in the diet was radically restricted, and the change in milling practice now converted flour from a vitamin B_1 liability into an asset. *National wheatmeal* (85 % extraction) contains approximately 1·3 mg. of vitamin B_1 per pound, as compared with pre-war white flours with about 0·5 mg. per pound.

Vitamin C

As a result of the shortage of ships in 1940, it was decided to dispense with imports of fruit, which form a bulky and wasteful cargo, and to depend, instead, for vitamin C on potatoes and other vegetables. The area of land available for vegetable production was, of course, limited and, while considerations such as hardiness to the weather, cropping season, perishability and national taste were given some degree of weight in planning the type and amount of the vegetables to be grown, the principle which finally influenced the acreage for any vegetable was the nutritional value of each crop.

Vegetables are perishable commodities and the yield from them often varies widely from season to season. It is, therefore, very difficult to regulate their distribution with any degree of precision. In order to implement the nutritional policy of depending on potatoes and green vegetables for vitamin C and a proportion of vitamin A, the Government of the United Kingdom controlled prices. In addition, however, they gave every encouragement to the domestic production of vegetables in gardens and allotments. Much publicity and quite detailed instructions were issued so that the individual gardener might make the best use of what little land was available to him. These domestic instructions, in parallel with the national directions to the farming community, were devised to produce supplies of vitamin C and vitamin A all the year round.

Vegetables, while they can provide adequate amounts of vitamin C in the diet of an adult, must be eaten in comparatively large quantities if they are to do so. They are not, therefore, suitable as the sole sources of the vitamin for infants and young children. In order to supply this nutrient for children under 5 years old, a scheme was put into operation in 1940 for using a substantial proportion of the national crop

of blackcurrants [*Ribes nigrum*], which are fruit exceptionally rich in vitamin C, for the preparation of a syrup for distribution as a ration to children. In the following year, concentrated orange juice, which was received from America under Lease-Lend, was substituted for blackcurrant syrup. These vitamin-C concentrates were issued to expectant mothers as well as to young children.

Vitamin D

Vitamin D is of special importance to expectant and nursing mothers, infants and children. As milk is one of the foods containing vitamin D, the allocation of milk to these groups of the population helps to provide part of their needs. Vitamin D is present in butter; at the beginning of the war it was, consequently, put into margarine at the level of 1 international unit per g. Later, in 1941, when eggs, which are also good sources of vitamin D, became scarce, the amount added to margarine was doubled to 2 international units per g. It has remained at that level, although the egg situation was improved by the importation of dried egg.

Besides the supply of vitamin D from foodstuffs, it was decided to make special provision of the vitamin for mothers and infants in the form of a standardized preparation of cod-liver oil. The original specification was 100 international units of vitamin D per g. Later, this was raised to 200 international units per g. More recently, in an effort to increase the proportion of women taking advantage of these supplements, an alternative issue to pregnant women has been in the form of tablets each containing 4000 international units of vitamin A, 800 international units of vitamin D and 250 mg. of calcium phosphate B.P., the recommended dose being 1 daily.

Communal Feeding

Communal meals have proved to be an essential adjunct to the country's nutritional policy. Their first importance appeared at the end of 1940 and the beginning of 1941, when many of the cities of Britain first came under heavy bombardment. The *British Restaurants* which were developed at that time are still of importance for providing meals in places to which large populations have come as the result of the war, and also to feed people in towns where domestic life may have been made difficult or where catering facilities are inadequate.

Hand-in-hand with communal meals intended for the population at large, has been the development of meals in factories. The importance of industrial restaurants was realized early in the war and in 1940 the Ministry of Labour issued an order, compelling all firms which employed more than 250 people and which were doing war work to establish an adequate restaurant. Within two years, more than 8,000 factory restaurants were set up. There are now over 16,000. Before the war there were only between one and two thousand industrial restaurants. Finally, out of the total 4½ million of the nation's schoolchildren, about 1½ million receive hot school-meals.

Food is allocated to restaurants, in proportion to the number of meals served, in a manner similar to the general system of distribution. Rations, "points" and, for milk, eggs and some other foods, controlled supplies, are made available. The amounts of food provided, however, are not equal for all types of restaurants but are varied in accordance with nutrition policy. Three levels of allocation have been fixed. *British Restaurants* receive amounts equal to those permitted for commercial restaurants and hotels; factories where moderately active work is done get more, and very heavy workers receive more still. The physiological needs of children are in many ways proportionally greater than those of adults; the allowances to school restaurants are, therefore, the largest of all. Besides rationed food, restaurants can, of course, buy what they please of bread, potatoes, vegetables and other items, the supplies of which are unrestricted.

In a short article such as this it is impossible to go into the full detail of all that has been done to guide Britain's food policy during the war. It is, however, perhaps fair to say that from all points of view, whether it be economy in transport or the health and well-being of the population, the application of scientific nutritional principles to the national administrations has in general been amply rewarded with success.

REVIEW OF SELECTED PAPERS

Assay of Vitamins in Food and Body-Fluids

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THE VITAMIN B_1 CONTENT OF NATIONAL FLOUR AND BREAD—THE RESULTS OF COMPARATIVE TESTS BY VARIOUS METHODS: With an Appendix on the Thiochrome Method for Estimating Vitamin B_1 in National Flour and Bread

by R. A. Peters, H. Chick, K. H. Coward, L. J. Harris, B. S. Platt & T. F. Macrae, *Biochemical Journal*, **37**, 433–439, September 1943

The writers of this paper comprised the Vitamin B_1 Sub-Committee of the Accessory Food Factors Committee, appointed jointly by the *Medical Research Council* and the *Lister* Institute. The vitamin-B_1 content of 85 %-extraction flours and of the bread made from one of the flours was determined by 15 laboratories and groups of investigators, in order to compare the merits of chemical with those of biological and microbiological assays. The bread was baked in a batch of forty loaves of two pounds [about 0·9 kg.] each by a usual commercial procedure. The loaves were sliced a few hours after baking and the slices were placed on slatted shelves and dried at 37° C. Drying was complete in two days, when the whole was powdered in a mill.

The biological methods used were the rat-growth method (Chick & Roscoe, 1929; Harris, 1940–1), the rat-bradycardia method and the "cure of convulsions" method (Harris, 1940–1). The microbiological method was that of Schultz, Atkin & Frey (1942). The chemical methods used were the thiochrome method [see below] and the azo method (Platt & Glock, 1943). All methods of estimation gave values which were in good agreement, especially for the flours. The chemical tests appeared to show a loss of the vitamin on baking, but the biological tests were not accurate enough to confirm this. No distinction was possible between the accuracy of the visual method as opposed to the photoelectric method in the determination of thiochrome, nor was the azo method more accurate than the thiochrome method. National flour (85 % extraction) contains between 3 to 4 μg. vitamin B_1 per g. of dry flour.

Appendix

Two methods for estimating vitamin B_1 in national flour and bread are described in detail. The method employing a visual comparison of fluorescence is based on that of Harris & Wang (1941) and Wang & Harris (1942). The photoelectric method is that of Booth (1940, 1942). Flour or bread is heated with 0·02*N* hydrochloric acid on a boiling water-bath for 10 minutes, sodium acetate-acetic acid buffer (*p*H 4·0) is then added, the mixture cooled to 40° C., brought to *p*H 4·0 and digested with takadiastase and papain for some hours. Fluorescent impurities are removed by washing with *iso*butanol at *p*H less than 4·0. The vitamin B_1 which remains in the aqueous layer is oxidized to thiochrome, a blue fluorescent substance, by potassium ferricyanide in the presence of methanol and excess sodium hydroxide. Simultaneously an equal volume of the flour extract is treated in

the same way, but without the addition of potassium ferricyanide; this forms the blank. A third flask is also set up containing a known amount of vitamin B_1 which is converted to thiochrome.

After treatment with hydrogen peroxide, the fluorescent material is extracted with *iso*butanol, the extract washed with water and clarified by the addition of ethanol. Equal volumes of the *iso*butanol extract of the " unknown " and the " blank " are compared visually in front of the Wood's glass window of an ultraviolet lamp. The standard " control " *iso*butanol extract is added to the " blank " from a graduated pipette until the fluorescence observed on looking down the tube matches exactly that of the unknown. The tubes are mixed by inversion and, before the final matching, a volume of *iso*butanol equal to that of the " control " *iso*butanol extract added to the blank is added to the unknown. The use of a Wratten 18A filter with the Wood's glass reduces errors due to substances with fluorescences of tints other than the purplish-blue tint of thiochrome. The matching must be effected rapidly.

When the Spekker fluorimeter is used, the standard should be stronger than the unknown. Because thiochrome is relatively unstable in ultraviolet radiation, 1 μg. quinine sulphate per ml. 0·1*N* sulphuric acid is used as the standard solution. The instrument is calibrated against the quinine standard by means of a series of aneurin standards, which have been prepared by oxidizing standard solutions of aneurin with precisely the same technique as is employed for flour or bread.

Identical laboratory conditions, particularly as to temperature, should be secured for the conversion of vitamin B_1 to thiochrome in both standard and unknown solutions. Test-tubes used in the visual method must be of non-fluorescent glass and identical in shape and of uniform diameter. To avoid the addition of fluorescent impurities, solutions must not come into contact with " unextracted " corks, rubber or tap grease. Glass-distilled water should be used. Solutions of thiochrome should be kept away from bright light and comparisons of fluorescence should be made in a darkened room.

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[1] [*see BMB* **494**]

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AN AZO COLOUR REACTION FOR THE DETERMINATION OF VITAMIN B_1 IN NATIONAL FLOUR

by B. S. Platt & G. E. Glock, *Biochemical Journal*, **37**, 439–443, 1943

This method which, with minor modifications, can be used for the estimation of pure vitamin B_1, has been devised by two whole-time members of the staff of the *Medical Research Council* working in the Nutrition Building of the *National Institute for Medical Research*, London. Flour (25 g.) is extracted with 0·5*N* hydrochloric acid (100 ml.) for several hours, stirring at intervals and allowing to stand overnight. All the vitamin passes into the aqueous phase, and is separated by decantation and filtration through a No. 1 Whatman filter paper. 40 ml. of the filtrate are run into a 150 ml. glass-stoppered bottle, 50 mg. of " Superfiltrol " (activated clay) are added and shaken for 30 minutes. The contents of the bottle are transferred to a 50 ml. centrifuge tube, the bottle being washed out with 5 ml. 0·5*N* hydrochloric acid.

Centrifuge until the clay is well packed, pour the supernatant fluid back into the bottle and repeat with another 50 mg. clay. Transfer the clay quantitatively to the centrifuge tube, discarding the supernatant fluid after centrifugation and wash the bottle with five successive portions of 5 ml. 0·5*N* hydrochloric acid. Resuspend the clay in the washings, centrifuge and discard the supernatant liquid. The washed adsorbate is suspended in 1 ml. distilled water, 2 drops of bromocresol-green are added and the *p*H is adjusted to 5 by addition of 2 % sodium hydroxide.

The suspension is transferred to a 25 ml. glass-stoppered cylinder. Wash the centrifuge tube with five successive portions of 2 ml. 60 % ethanol, adding the washings to the cylinder. 2 ml. of 3 % sodium bicarbonate in 0·6*N* sodium hydroxide in a dry test-tube are treated with 2 ml. of diazo reagent. The diazo reagent is prepared by adding 1·5 ml. 4·4 % sodium nitrite solution to 1·5 ml. 0·8 % *p*-aminoacetophenone in 3·5 % hydrochloric acid, both solutions having been cooled to 0–5° C. and kept at that temperature for 10 minutes, when a further 6 ml. sodium nitrite solution is added.

The solution is made up to 50 ml. with ice-cold distilled water and allowed to stand at least 15 minutes before use. It is prepared on the day of the test and must be kept at 0–5° C. After addition of the diazo reagent, mix by inverting, and exactly 1 minute later add to the suspension of the adsorbate in the cylinder. Mixing is effected by inversion every few minutes. After 20 minutes, 5 ml. of toluene are added and mixing by gentle inversion is continued at frequent intervals for a total period of 1 hour. The toluene layer is transferred to another stoppered cylinder containing 10 ml. 0·5*N* hydrochloric acid and washed by inversion. The toluene layer is then transferred to a 5 ml. centrifuge tube, a small amount of anhydrous sodium sulphate is added, the contents are mixed and rapidly centrifuged.

A standard solution is prepared by adding 1 ml. of a diluted standard vitamin B_1 solution to a second washed adsorbate, adding 2 drops of bromo-cresol-green indicator and proceeding as described above, except that 10 ml. of toluene are used to extract the dye. The two solutions are compared in a Duboscq type of colorimeter fitted with cups having a stem capacity of 5 ml. For the estimation of solutions of pure vitamin B_1, the preliminary adsorption on activated clay is omitted, but it was found necessary to wait for 60 minutes instead of 20 minutes before extracting the dye with toluene.

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NICOTINIC ACID AND RIBOFLAVIN IN BEEF EXTRACTS AND CORNED BEEF

by R. G. Booth & E. C. Barton-Wright, *Lancet*, **1**, 565–567, 29/4/44

These workers in the Ministry of Food Cereals Research Station, St. Albans, have determined the nicotinic acid and riboflavin contents of meat extracts, meat juices, fresh and corned beef, and samples of beef and pickling liquors, during processing as corned beef. Nicotinic acid was estimated by the method of Kodicek (1940), modified in some instances by the replacement of 0·4 ml. of *p*-aminoacetophenone reagent by 1 ml. of 10 % procaine in 10 % HCl, in order to obtain a more stable and intense colour. Riboflavin was estimated by the microbiological method of Barton-Wright & Booth (1943).

Five meat extracts contained from 375 to 1,025 μg. nicotinic acid and 15·6 to 25·8 μg. riboflavin per g. Meat juices gave a range of 345 to 615 μg. nicotinic acid per g.; *marmite* yeast-extract contained 655 μg. nicotinic acid per g. Eight samples of corned beef contained from 8·5 to 33·4 μg. nicotinic acid and 0·7 to 1·85 μg. riboflavin per g. as purchased. Fresh beef (six different cuts), as purchased, contained 46 to 71 μg. nicotinic acid per g. and roast beef sirloin 57 μg. per g. The low content of corned beef is chiefly due to losses by elution during pickling. It is not due to destruction by sodium nitrite, which is used in this processing.

An average " civilian " helping of corned beef—2 oz. [about 60 g.]—would yield 0·85 mg. nicotinic acid. The same serving of roast beef would give more than 4 mg. About 0·05 mg. riboflavin is provided by the corned beef as against 0·25 mg. in roast beef. A teaspoonful of meat extract, as used to make a large cupful of beverage, would have an average of 5 mg. of nicotinic acid and 0·25 mg. of riboflavin.

These results give an entirely new perspective as to the value of meat extracts nutritionally, and challenge the findings that such articles are of negligible food value but useful as flavouring agents and appetizers (*Lancet*, 1908).

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THE RIBOFLAVIN CONTENT OF FOOD SERVED IN ROYAL AIR FORCE MESSES

by T. F. Macrae, E. C. Barton-Wright & A. M. Copping, *Biochemical Journal*, **38**, 132–135, 1944

The authors report results of estimations of riboflavin content of total diet, using the method described by Macrae, Henry & Kon (1943) for obtaining from a representative mixed dietary a dehydrated, homogeneous product. From the average daily dry weight of the food served and the estimation of its riboflavin content, it was possible to determine the average daily intake of riboflavin per person. Estimations of riboflavin were performed (i) by the biological technique devised by El Sadr, Macrae & Work (1940) as modified by Copping (1943), (ii) the microbiological method of Snell & Strong (1939) as modified by Barton-Wright & Booth (1943). Experimental details are given.

Results by the biological method were usually rather higher than those obtained by the microbiological method but, in general, agreement was good.

Of the Royal Air Force personnel tested, airmen were found to have an average daily intake of about 2 mg. and airwomen about 1·8 mg. riboflavin. The optimum riboflavin requirement has been estimated (*Committee on Food and Nutrition*, 1941) as 3 mg. for active men and 2·5 mg. for active women. Other findings, however, indicate that these values may be too high. Further, Lyle, Macrae & Gardiner (1944) observed no sign of riboflavin deficiency in R.A.F. personnel stationed in Britain, and this tends to support the view that the riboflavin intake, as determined by the present authors, was adequate. Regular consumption of beer, which has recently been shown to be a surprisingly good source of riboflavin (Barton-Wright, Moran & Sarson, 1943; Hopkins, 1943), would materially supplement dietary riboflavin, but it is estimated that not more than 10 % of R.A.F. personnel are regular beer drinkers.

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[1] [*see BMB* **523**] [2] [*see BMB* **519**]

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A STATISTICAL EXAMINATION OF THE ACCURACY OF VITAMIN A ASSAYS: AN ANALYSIS OF THREE CO-OPERATIVE EXPERIMENTS DESIGNED TO ASCERTAIN THE VALUE OF THE CONVERSION FACTOR FOR TRANSFORMING SPECTROPHOTOMETRIC VALUES INTO INTERNATIONAL UNITS

by J. O. Irwin, *Journal of Hygiene*, **43**, 281–314, April 1944

Those interested in biological assays will certainly be conversant with the author's exposition in *Statistical methods applied to biological assays* (1937), in which he reviewed the best methods available at that date. In the article under notice, the author, a member of the statistical staff of the *Medical Research Council*, gives the results of three experiments devised to re-examine the conversion factor of 1600 which had been previously allotted by the 1934 conference on Vitamin A standardization for converting the results of spectrophotometric tests for vitamin A into international units. Three experiments were devised using (i) halibut-liver oil, (ii) a U.S.P. reference oil, and (iii) solid vitamin A 2-β-napthoate.

A report on the first experiment had already been published (Hume, 1937) providing no support for changing the existing value, but in view of the fact that the results submitted from the different laboratories had been calculated by different methods, Dr. Irwin here gives the results of a revised calculation. The conversion factors obtained from the three experiments were respectively 1570, 1820 and 1770, and as these did not significantly differ they were consistent with the hypothesis that the same conversion factor holds for all three substances. The results were therefore pooled, and a conversion factor of 1740 with limits of error of 93–107 % or 1620–1860 obtained.

More important to workers in this field of statistics than the actual results, is the opportunity which the analysis has given to the author to discuss and illustrate the greater accuracy in biological and other assay results now obtainable by the improvements which have been evolved in this branch of statistical analysis since his review of the methods existing in 1937.

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498

APPARENT VITAMIN C IN FOODS

by F. Wokes, J. G. Organ, J. Duncan & F. C. Jacoby, *Biochemical Journal*, **37**, 695–702, 1943

It has long been known that 2:6-dichlorophenolindophenol reacts with a number of substances besides ascorbic acid. During the past three years, these workers in the *Ovaltine* research laboratories have accumulated evidence to show that, under certain manufacturing and storage conditions, the apparent vitamin-C content of foods can become considerably higher than the anti-scorbutic vitamin-C value. They used both the visual dye titration method and the potentiometric method of Harris, Mapson & Wang (1942).

The apparent vitamin C is determined after treatment with formaldehyde at *p*H 4·5 for 6 minutes, which destroys all the true vitamin C, adding an excess of metaphosphoric acid solution to bring the *p*H to 1·0 and titrating with the dyestuff. The total vitamin C is determined with similar timing and rate of addition of dye, without the addition of formaldehyde.

The highest yields of apparent vitamin C are obtained when the materials (potatoes, cabbage, carrots, germinated grains, grass and lucerne) are treated with 2 % (volume/volume) of HCl or H_2SO_4 on a water bath at 90–95° C., or occasionally on a sand bath for upwards of 8 hours. If smaller concentrations of acid are used, the rate of production of apparent vitamin C is much slower. In 0·25 % HCl apparent vitamin C is unstable to prolonged heating. Heating alone, without the addition of acid, produces an increase in the apparent vitamin-C content of malt extract, fruit syrups and other products, which may mask the destruction of true vitamin C if present.

Apparent vitamin C may be gradually formed during storage under normal conditions. The percentage increase in apparent vitamin C is highest at raised temperatures and lowest in nitrogen-filled containers when all but 1–2 % of the total moisture has been removed. Apparent vitamin C occurs in cocoa and chocolate from which it is not removed entirely by precipitation of the tannin.

In fresh fruit, usually less than 10 % of the total vitamin C occurs as apparent vitamin C, except for unripe walnuts where it may reach 80 %. Prolonged storage, or the heating of fruit juices, may produce a great increase of apparent vitamin C—to form eventually the greater part of the total vitamin C as determined by the visual dye titration. Demerara sugar, some table syrups, molasses and dark beers may contain apparent vitamin C. Whilst the common raw vegetables do not appear to contain significant amounts of apparent vitamin C, it may be produced during roasting and frying and it does occur in such herbs as sorrel and parsley.

Apparent vitamin C is formed as rapidly at *p*H 4–5 as at *p*H 1·0 and is best preserved in nitrogen-filled containers kept in the dark, or by use of cyanide. It is destroyed by ascorbic-acid oxidase, or by treatment with 0·1 % hydrogen peroxide or sodium percarbonate at 37° C. for 2–3 minutes. It is gradually destroyed by ultra-violet irradiation. A malt extract having a high concentration of apparent vitamin C but possessing no antiscorbutic action showed a distinct

spectroscopic maximum at about 280 mμ. A discussion of the possible relationship of apparent vitamin C to reductone, reductic acid, dihydroxymaleic acid and hydroxytetronic acid is appended.

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499

ESTIMATION OF "TRUE" ASCORBIC ACID IN URINE

by D. Richter & P. G. Croft, *Lancet*, **1**, 802–803, 26/6/43

The method of estimating vitamin C in urine by titrating with dichlorophenolindophenol, developed by Harris & Ray (1935), is liable to error owing to the presence in the urine of sodium thiosulphate and possibly other substances which decolourise the dye. Although a number of modifications of the technique have been proposed to eliminate this error, doubt has been expressed as to whether the figures obtained after their application can be regarded as representing "true ascorbic acid."

The present authors, writing from the *London County Council* Pathological Laboratory and the *Mill Hill* Emergency Hospital, describe a simple and rapid lead precipitation procedure for eliminating the interfering substances in urine which appears satisfactory, and which gives results similar to those obtained by Neuweiler's (1936) ascorbic oxidase method.

In the collection of urine samples it was found that the use of metaphosphoric acid caused much less loss of vitamin C than acetic acid. For most estimations, 2–3 hour samples of fasting urine, or urine after an ascorbic acid-free breakfast, were collected by passing straight into bottles containing 10 g. metaphosphoric acid. Ten ml. of the urine, containing 5% metaphosphoric acid, were centrifuged after the addition of 1·5 ml. of lead acetate solution (25% $Pb(Ac)_2H_2O$). The precipitate could also be removed by filtering. Samples of the filtrate were titrated for ascorbic acid content by running the dye into the urine (the reverse of the usual procedure). This was done in order to avoid having present during the titration a large excess of dye, which might oxidise substances less rapidly oxidised than ascorbic acid.

The treatment with lead acetate not only removed interfering substances but also some of the colouring matter from the urine, and the end point of the titration was thus easier to determine. There was no loss of ascorbic acid added to the urine as a result of the lead acetate precipitation. Tests in which sodium thiosulphate or quinol were added to the urine showed that they were quantitatively removed by the lead acetate. Other possible interfering substances in urine such as glutathione, catechol, catechin, and sodium thiocyanate added to urine samples in concentrations up to 10 mg./100 ml. did not reduce the dye rapidly enough to interfere with the titration.

The observations made with the lead acetate precipitation method were tested against the ascorbic acid oxidase method. The oxidase was made from cucumbers, cauliflowers or broccoli after the method of Meiklejohn & Stewart (1941). Estimation of the total reducing activity was obtained by simple titration of the urine with indophenol. Then an aliquot portion of the same sample was treated with ascorbic oxidase in the presence of oxygen. Subsequent titrations gave the amount of reducing substances other than ascorbic acid in the treated urine. The difference between the first and second figures gave the amount of reduction due only to ascorbic acid.

The figures for true ascorbic acid obtained by the lead precipitation method and the ascorbic oxidase method agreed very closely.

Aspirin increased the excretion of reducing substances in the urine, but it was not possible to find any evidence as to whether the increase was due to "true ascorbic acid" or to other phenolic reducing substances.

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THE ESTIMATION OF 'TRUE ASCORBIC ACID' IN BLOOD

by D. Richter & P. G. Croft, *Biochemical Journal*, **37**, 706–708, 1943

The method which has been evolved by these authors is as follows: 4 ml. of venous blood (showing no hæmolysis and containing 0·3 g. sodium oxalate per 100 ml. blood) are transferred to a 15 ml. centrifuge tube containing 0·05 ml. *sec*-octanol, and coal gas is bubbled through. 4 ml. distilled water are added and the gassing is continued for ten minutes. Freshly prepared 32% metaphosphoric acid solution (2 ml.) is added, followed after mixing by 1 ml. 25% lead acetate solution and 2 ml. saturated sodium acetate solution. After thoroughly mixing, the suspension is centrifuged and 2 ml. portions of the supernatant fluid are titrated with a 0·002% solution of 2:6 dichlorophenolindophenol which has been standardized against a 0·0005% solution of ascorbic acid in 2% metaphosphoric acid. The end-point is reached when a faint pink colour persists for ten seconds and matches a solution made by adding 0·05 ml. of the dye solution to 1 ml. of 1% metaphosphoric acid solution contained in a similar vessel. In the calculation, the amount of dye added to the blank is subtracted from the burette reading and a correction is made for the ascorbic acid contained in the protein precipitate.

Results obtained using this method agree more closely with those obtained with the ascorbic-acid oxidase method than those obtained without treatment with lead acetate. No significant loss of ascorbic acid occurs in whole blood stored at room temperature, with or without occasional shaking, for 24 hours. The maximum loss in ten specimens was only 22% in 48 hours. The blood ascorbic acid of 350 normal factory workers in the Birmingham area varied from 0·15–0·85 mg./100 ml. with a mean value of 0·25 during May–July 1943.

Studies on Vitamin C *

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COMPLEMENT ACTIVITY AND VITAMIN C

by E. Kodicek & B. Traub, *Biochemical Journal*, **37**, 456–460, 1943

In recent years conflicting reports as to the correlation of complement activity and vitamin-C intake have been published. The question has been re-examined by E. Kodicek working for the *Medical Research Council* in the *Dunn* Nutritional Laboratory and B. Traub in the Department of Pathology in the University of Cambridge. The end-point of the complement titration was taken as the volume of undiluted serum required to produce a 50% hæmolysis in a standardized system.

Sheep erythrocytes from a single source were used. Blood samples (2–3 ml.) were obtained by cardiac puncture without anæsthesia, and the serum was diluted 1:30 with 0·9% buffered saline of *p*H 7·4. Eight tubes containing different amounts of diluted serum were tested against 0·5 ml. of a suspension of sensitized sheep erythrocytes containing 50 mg. hæmoglobin per ml. After incubation at 37° C. for 30 minutes the tubes were cooled to 2–4° C., 2 ml. of buffered saline added, and after centrifugation the percentage hæmoglobin in the supernatant fluid was determined colorimetrically.

Two series of experiments were made, one during June–July on 22 guinea-pigs weighing 300–368 g., the second during October–December on 18 animals weighing 440–665 g. The basal diet of 11 of the first group of animals was supplemented with 5 mg. of ascorbic acid plus 15 g. cabbage, in the second series supplements of 0·5, 1·0 and 10 mg. of ascorbic acid were given daily. The complement titres of the deficient animals in the summer series ranged from 50–81 units with a mean of 61 units in the second and 63 units in the third week of the diet, whilst those on the supplemented diet were 51–72 units with an average of 60 units. In the winter series the average titre after a daily dose of 0·5 mg. ascorbic

* [*see also BMB* **498, 499, 500, 521, 534, 535, 536, 537, 539, 547**]

acid for four weeks was 69 units, which fell to 53 after the supplement had been increased to 10 mg. ascorbic acid daily for five weeks. When the animals had an initial supplement of 10 mg. ascorbic acid daily for four weeks the average titre was 61 units, which became 80 units when the daily supplement was changed to 0·5 mg. ascorbic acid for the succeeding five weeks.

A statistical examination of the results showed without doubt that, when a reliable method of complement titration is used, there is little if any difference between the complement titres of individual guinea-pigs within a group, whether they are maintained on a deficient or optimal vitamin-C diet.

502

SURVEY OF VITAMIN-C LEVEL IN WARTIME IN PREGNANT WOMEN

by A. A. Craig, F. J. W. Lewis & D. Woodman, *British Medical Journal*, **1**, 455–457, 1/4/44

This paper, by the Assistant Medical Officer of Health of Bristol and workers in the Department of Preventive Medicine in Bristol University, records the urinary excretion of ascorbic acid, during the 2nd–4th hours after the ingestion of 70 mg. of ascorbic acid per 10 stone [about 64 kg.] body weight, at monthly intervals, in 40 pregnant and 5 non-pregnant women from May 1942 to February 1943. Of the 40 pregnant women, 3 had spongy gums and an anæmia, 6 spongy gums alone, and 8 anæmia alone. Wide fluctuations in the quantity of ascorbic acid excreted were observed, although average values disclosed a significant seasonal variation.

The average value of the urinary ascorbic acid after the test dose for the 5 controls for the whole period (48 tests) was 43·5 mg./100 ml.; whereas that for the 40 pregnant women was 39·7 mg./100 ml. The average excretion of the test dose after the 28th week of pregnancy was slightly smaller than that reached during the first 28 weeks. The authors concluded that the degree of saturation in both groups was low, although during June to October the degree of saturation rose and the diet appeared to be more adequate.

503

THE ASSESSMENT OF VITAMIN-C NUTRITION IN MAN

by F. T. G. Prunty & C. C. N. Vass, *Biochemical Journal*, **37**, 623–629, 1943

The need for a reliable and simple assessment of the nutritional status with respect to vitamin C has been emphasized by the importance which has been ascribed to its role in wound-healing and by the possibility of a diminished supply of vitamin-rich foodstuffs in war-time diets. This paper which comes from the Departments of Pathology and Physiology, *St. Thomas's* Hospital Medical School, London, shows that under certain conditions a single determination of the plasma ascorbic acid can be used to assay the standard of vitamin-C nutrition. The report is compiled from the data obtained from 50 subjects (hospital patients and staff) and correlates the results of urinary saturation tests and estimations of plasma ascorbic-acid concentration with an assessment of the nutritional state of the subject with respect to vitamin C.

Urines were collected in dark-coloured Winchester bottles [which have a capacity of about 2·27 l.] containing 70 g. metaphosphoric acid and the ascorbic acid and dehydroascorbic acid contents were determined in the usual way. A standard dose of 700 mg. of ascorbic acid per 140 pounds [about 64 kg.] body-weight was given orally each day. The dose was administered in 50 mg. tablets, as nearly as possible in two equal portions, one at the commencement of each 24-hour collection of urine, the other approximately 12 hours later. This was done in order to minimise the effect of the renal overflow. Blood samples were taken, either at the end of the 24-hour period before the first dose of the subsequent 24 hours, or, if no dose was given, 3 hours after taking food. The plasma ascorbic-acid concentration was determined by direct visual titration of a protein-free filtrate with 2:6 dichlorophenolindophenol.

The behaviour of the plasma ascorbic acid during an extended saturation of the subject with ascorbic acid was observed in nine subjects. Whilst very marked fluctuations occurred in the urinary excretions, the plasma ascorbic-acid concentrations remained constant. Moreover, if in the same subject the urinary saturation test was repeated, it was found that when the plasma ascorbic-acid content was low, the rise in the daily excretion of ascorbic acid was relatively slow, and no point of sharp demarcation was obvious. If the plasma ascorbic-acid concentration initially was above 0·2 mg. per 100 ml. a spectacular rise in the urinary excretion of ascorbic acid was readily observed and the day on which "saturation" was reached was easily interpreted. Because saturation was attained in some of their subjects who excreted less than 50% of the test dose during 24 hours, the authors have arbitrarily defined "'saturation' as the excretion of 33⅓% of the test dose based on 700 mg. per 140 pounds body-weight and administered as outlined above."

A figure (III) in this paper shows the relationship between the plasma ascorbic-acid concentration before the saturation test and the dose of ascorbic acid required to bring the subject to a "state of saturation" (18 subjects). At the "state of saturation," the administration of the test dose of 700 mg. per 140 pounds body-weight would result in an excretion of ⅓ of the test dose in the urine in the next 24 hours. The plasma concentration at the "state of saturation" is 0·80 mg. ascorbic acid per 100 ml. The relationship between the two variables is not linear, which may account for some of the divergence of opinion as to the validity of the plasma ascorbic-acid content as an index of vitamin-C nutrition. The maximum charge in the dosage of vitamin C required to produce "a state of saturation" at a given plasma level occurs at a value slightly less than 0·4 mg. per 100 ml. If the initial plasma ascorbic-acid concentration is 0·4 mg. per 100 ml., 450 mg. of ascorbic acid per 140 pounds body-weight will bring the subject to a "state of saturation" which is demonstrated by the further ingestion of 700 mg. on the succeeding day. This is equivalent to a good saturation-response in two days with Harris's urinary saturation test (Harris, 1943).

During the progress of this work 8 subjects were kept for varying periods on controlled intakes of ascorbic acid for known periods. It was found that if the subject has previously been on a higher intake of ascorbic acid and reverts to a lower intake, the plasma ascorbic-acid concentration tends to remain at a higher level than that reached when the procedure is reversed, unless a considerable period on the lower intake is maintained (probably at least 30 days).

The authors conclude that the plasma ascorbic-acid concentration is in man an index of the nutritional state with respect to vitamin C, unless, within the previous 30 days, the subject has been on an appreciably higher intake of ascorbic acid than he is receiving at the time of the test. A level of 0·8 mg./100 ml. represents a "state of saturation" and evidence is presented for the desirability of a plasma ascorbic-acid concentration of not less than 0·4 mg. per 100 ml., for which an intake of approximately 60 mg./day is necessary.

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[1] Harris, L. J. (1943) *Lancet*, **1**, 515

[1] [*see BMB* **107**]

504

VITAMIN-C NUTRITION IN A HOSPITAL: With Observations on a Case of Scurvy

by F. T. G. Prunty & C. C. N. Vass, *Lancet*, **1**, 180–182, 5/2/44

The concentration of ascorbic acid in the plasma of hospital in-patients and members of the medical and nursing staffs was determined at various times over a year. Seventeen patients taken at random were examined in the period May to July 1942, and fifteen during September and October. In the first period only 6% would give a saturation response in 2 days to the normal urinary saturation test; the plasma ascorbic-acid concentration averaged 0·12 mg. per 100 ml. with a range of 0·00 to 0·30. In the second period, 27% of the subjects would have given a positive urinary saturation response in 2 days, as indicated by an average plasma ascorbic-acid concentration of 0·24 mg. per 100 ml. with a range of 0·03 to 0·65. Comparison of the plasma values of the male members of the medical staff showed the same seasonal variation and emphasized the poorer nutrition of the hospital in contrast with the uniformly higher figures found among the non-resident staff. A small group of nurses examined in

February 1943 supplied further evidence of the relative poverty of the hospital diet in regard to vitamin C, whilst a corresponding group of student nurses who resided in a hostel had satisfactory plasma ascorbic-acid levels.

The latter half of the paper contains the results of an extended ascorbic-acid saturation test on a scorbutic mechanic aged 27 years, weighing 131 pounds [about 60 kg.]. He was admitted to hospital on 30/5/42 complaining of rash on his shins for one month, painful swelling of the left forearm and leg for one week, and swelling of the legs for two days. He had a hæmatemesis 2 years previously, afterwards reverting to a normal diet; six months ago there was a sudden onset of gastric ulceration demonstrated radiologically. Since the ulcer had developed he had kept strictly to a diet of two pints [about 1·14 l.] of pasteurized milk, porridge, fish, dry toast, butter and chocolate daily. At examination his gums were edentulous, the soft palate reddened and slightly œdematous. The anterior aspects of both legs were covered with small petechiæ. Œdema of both ankles was marked and the left calf was somewhat swollen and tender. Ecchymoses were present on the inner sides of both ankles, in the right antecubital fossa and on the palmar aspect of the right wrist. The left arm had a fixed flexion of 160° with some tender swelling deep to the extensors of the left forearm. Hyperkeratotic papules were present on the outer sides of both thighs, across the lumbar region and over the dorsal surfaces of both upper arms. A blood count showed 4,390,000 erythrocytes, hæmoglobin 70% (Haldane), 5,830 leucocytes with a normal differential count and 280,000 platelets. After three minutes at 100 mm. Hg pressure, 30 petechiæ were produced in an area approximately 6 cm. in diameter in the left antecubital fossa.

After daily treatment with 750 mg. ascorbic acid in doses of 400 and 350 mg. at 12-hour intervals, the hyperkeratotic papules disappeared within 7 days and the petechiæ and ecchymoses had largely faded. After 18 days the capillary fragility test gave only 3 petechiæ. A prolonged saturation test was carried out on this subject and plasma ascorbic-acid concentrations were determined at intervals. The results attained were similar to those obtained in non-scorbutic subjects, whose initial plasma ascorbic-acid concentration was also 0·00 mg. per 100 ml. The scorbutic patient was saturated with a total dose of 4,300 mg. per 140 pounds [about 64 kg.] body-weight in 6 days.

Prunty & Vass (1943) have reported a patient with a gastric ulcer and an initial plasma ascorbic-acid concentration of 0·00 mg. per 100 ml. who was saturated in 8 days with a total dose of 4,900 mg. of ascorbic acid per 140 pounds body-weight, and this emphasizes the impossibility of diagnosing scurvy either by determination of the plasma ascorbic-acid or by the urinary saturation tests alone. Further, the failure to observe signs of sub-clinical scurvy in this survey substantiates the view that a sub-standard intake of vitamin C is not necessarily equivalent to a clinical deficiency (Harris, 1943).

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[1] [*see BMB* **107**] [2] [*see BMB* **503**]

505

SCURVY : A SURVEY OF FIFTY-THREE CASES

by R. B. McMillan & J. C. Inglis, *British Medical Journal*, **2**, 233–236, 19/8/44

This is a report by the medical superintendent and the late senior medical officer of cases of scurvy admitted to the medical wards of the *Eastern General* Hospital, Edinburgh, between August 1937 and February 1944. The patients were 48 males and 5 females whose ages ranged from 41 to 82 years. They formed 0·16% of the total medical admissions in 1937–38, an incidence which gradually rose to a maximum of 3·07% in 1941–42 and fell to zero in 1943–44, although the total number of medical admissions, which had fallen to one-third of the 1937–38 total in 1940–41, had almost reached the pre-war level in 1943–44.

Eighty-one per cent. of the patients were aged 65 or over, and 96% lived alone. More than 90% cooked for themselves. An analysis of their social and economic background showed that poverty was by no means the whole reason for the condition. The disease developed most commonly in June, then came May, March and April in that order. In 80% of cases the time elapsing between the first sign or symptom and admission to hospital was less than one month. The disease, by the usual standards, was mild in 2 instances and moderate in 51; all had skin petechiæ. Where teeth existed, spongy and infected gums were prevalent. The disease appeared first in one leg, then the other, and finally in the arms. Hyperkeratosis perifollicularis was very common but not universal. Three instances each of multiple neuritis and pellagra were also recognized clinically. Neither the positive-pressure cuff test of Hess (1920) nor of Göthlin (Falk, Gedda & Göthlin, 1932) were diagnostic or in alignment with the plasma ascorbic-acid concentration or the saturation requirements.

The vitamin-C content of the urine varied from 1·5 mg. to 16·0 mg., with an average of 7 mg. per 24 hours. Thirty subjects required between 2·0 to 5·9 g. (average 3·85 g.) of ascorbic acid to produce saturation. No steady relationship between the saturation requirements and the clinical extent of the disease was observed. The plasma ascorbic-acid concentration in 22 patients, within 48 hours of admission, varied from 0·00 to 0·29 mg. per 100 ml. There was a constant relation of plasma level to saturation requirements but not to the clinical picture.

A complete blood examination was made on 38 males and 2 females within 48 hours of admission. The hæmoglobin concentration varied from 5·3 to 11·9 g. per 100 ml. An examination of the sternal marrow was made on 6 subjects: 5 were normoblastic, two of these had a few megaloblasts, one was megaloblastic. No constant relationship was noted between the anæmia and the extent of the hæmorrhages, or the plasma ascorbic-acid level, or the saturation requirements of the patients. The anæmia was predominantly normocytic, rarely hypochromic microcytic, and more rarely macrocytic.

Groups of patients were placed on diets containing known quantities of iron with no vitamin C, or with 15 mg. vitamin C, or with a maintenance dose of 100 mg. vitamin C daily after saturation had been attained. The experimental periods lasted from 9 to 25 days. No patient on a vitamin-C-free diet showed a fall in either the erythrocyte count or hæmoglobin, but in most individuals the presence of the vitamin accelerated regeneration.

The authors ascribe three root causes to the production of this " bachelor " scurvy. (i) Ignorance, especially among males, of the need for potatoes and vegetables in the diet. (ii) Apathy, producing absence of the same items because they required preparation and cooking. (iii) Poverty, which made it impossible to buy an adequate diet or to obtain lodgings with adequate cooking facilities.

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Water Metabolism

506

EFFECTS OF DRINKING SMALL QUANTITIES OF SEA-WATER

by W. S. S. Ladell, *Lancet*, **2**, 441–444, 9/10/43

The work described in this paper was part of a study of the physiology of persons receiving the same rations as shipwrecked personnel. It was performed on behalf of the committee on the care of shipwrecked personnel of the *Medical Research Council*. The subjects were given the very limited diet which was at that time issued to lifeboats, and consisted of 30 g. of biscuits, 30 g. of sweetened condensed milk, 30 g. of butter or margarine and 30 g. of chocolate per day. This food did not supply the men with enough calories to meet their physical needs and it provided less than 1 g. of salt daily.

Four experiments were carried out, the object of all of them being to study how men reacted when they were given (*a*) no water, (*b*) a limited amount of fresh water, or (*c*) sea-water.

180 cm.³ of sea-water were usually given each day, but up to 400 cm.³ were administered to one person. In all some 25 subjects seem to have been studied. It was found that when the men were getting too little water to satisfy their needs, but were not taking sea-water, they continued to pass 350 to 450 cm.³ of urine per day, and that the volume passed was not related to the severity or duration of the dehydration. The concentration of urea in the urine was high and values up to 6 % were found. The urine contained much salt on the first day of water deprivation—which was to be expected as the subjects had until then been taking normal quantities of salt—but thereafter the losses of salt became very much lower.

A diuresis occurred in all experiments in which the subjects drank sea-water, and the excretion of salt remained high or rose if it had been low. The output of water did not rise (on average) by more than the amount of sea-water ingested. There was some evidence that the end-products of N metabolism were more freely excreted when the urine volumes were raised by the sea-water, and the author concludes that these quantities of sea-water diminished or prevented N retention.

The author considers that, in addition to these physiological effects, there was a definite psychological value in having additional fluid to drink even if it was salt to the taste. However, in a footnote to this paper, Dr. B. S. Platt of the *Medical Research Council* points out that, taking into consideration other relevant evidence, the drinking of sea-water was *not* recommended.

507

A STUDY OF DEHYDRATION BY MEANS OF BALANCE EXPERIMENTS

by D. A. K. Black, R.A. McCance & W. F. Young, *Journal of Physiology*, **102,** 406–414, April 1944

The work described in this paper was carried out at the Department of Medicine, Cambridge, to study man's reactions to a deficiency of water. Two men, one of them an author of the paper, drank no fluids for three and four days respectively, and maintained their normal calorie intake by eating food containing very little water, such as biscuits, butter, jam, cheese and bread.

On this régime the men lost weight, and by the third or fourth day it became very difficult indeed to swallow the dry food. Their voices changed slightly, their faces became rather sunken and pale, and their lips cyanosed. It was evident that their efficiency was impaired. These changes were observed in other subjects and are considered to be characteristic of water deprivation. This general appearance of ill-being vanished within a few hours of the restoration of fluid.

Changes in the circulating fluids were investigated, and determinations of nitrogen and mineral balance were carried out on these two men. It was found that in spite of a loss of weight which amounted to nearly 4 kg.—most of which was water—there was practically no rise in the plasma proteins, or in the hæmatocrit. It was evident that the volume of the blood had been maintained at the expense of the fluid in some other compartment of the body. The sodium in the serum rose by about 10 % and the potassium fell. The urea was raised to double its normal level. Dehydration led to an increased production of urea within the body, and it is suggested that this was due to a breakdown of tissue protein. The output of nitrogen fell on the first day of dehydration, due to the fall in the volume of urine, but rose on subsequent days as the level of urea in the blood increased. The men lost about 3,500 cm.³ of their body water and it is estimated that about ⅓ of this came from the extracellular fluids, the remainder from the fluid within the cells.

Although both subjects excreted about as much sodium as their food contained, they excreted none of the sodium corresponding to the extracellular fluids which they lost. In spite of the fall in the serum potassium, the men excreted rather more potassium than they took in with their food, but again, not enough to prevent a rise of potassium in their cellular fluids. Consequently the osmotic pressure of the whole body rose, and, in the light of some experiments which were carried out by Kerpel-Fronius & Leövey in 1929 and 1931, the authors consider that a retention of osmotically-active substances within the body must be seriously considered as the ultimate cause of death in prolonged dehydration.

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508

THE SECRETION OF URINE DURING DEHYDRATION AND REHYDRATION

by R. A. McCance & W. F. Young, *Journal of Physiology*, **102,** 415–428, April 1944

This paper deals with the reactions of the kidney to a shortage of water within the body, and the work was done in conjunction with that reported by Black, McCance & Young (1944). Twelve persons were dehydrated, ten men and two women, and the method used was similar to that described by Black *et al.* It was first shown by experiments on four people that dehydration, sufficiently prolonged to make the subjects lose 4–7 % of their body-weights, made little difference to the inulin and diodone clearances, i.e. to the glomerular filtration-rates and to the effective blood flow through the kidney. Hence the urinary changes which characterize dehydration of this degree of severity must be essentially the results of changes in tubular activity.

The authors next tried the effect of giving large doses of urea and of sodium and potassium chloride to persons who had been deprived of water for some days. These substances were given as hypertonic solutions. One person took 500 cm.³ of 3·6 % sodium chloride by mouth, and another was given 350 cm.³ of 10 % urea intravenously. The effects produced in the dehydrated subjects were not unlike those produced in the same persons when they were normally hydrated. The flow of urine was increased, and the diuresis produced by sodium and potassium chlorides raised the urea clearances. The osmotic pressure of the urine fell somewhat as the volume rose, and the osmotic pressure of the body must have been increased. One interesting effect followed the administration of the urea during dehydration. The serum chloride fell from 391 to 359 mg. per 100 cm.³ and there was a profound fall in the excretion of this ion.

Balance experiments were also carried out, and it was shown that the volume of the urine passed during the days of dehydration depended largely upon the quantity of salt in the food. The volumes of urine, however, passed on the second, third and fourth days of water deprivation were no smaller than those passed on the first day. In diets containing some 12–15 g. of salt, the output of water by the kidney ranged from 633 to 815 cm.³ in the 24 hours. When the salt intake was reduced to 2–5 g. per day, the daily urine output fell to 365–413 cm.³ As the high salt diets contained no more water than the low salt diets, the additional water excreted must have come from the already depleted body-water, and it is evident that a diet containing very little salt would enable persons on a reduced water intake to conserve 300 to 400 cm.³ of their precious store of body-water every day.

When persons, who had been deprived of water for some days, and who had been taking 5–15 g. of salt, were rehydrated in stages, the urine volumes fell, the percentage of chlorides in the urine fell, and the percentage of urea rose. Thus one subject, who had been passing 605 cm.³ of urine per day on a water intake of 470 cm.³, reduced his urine volume to 506 cm.³ when he was given 3804 cm.³ of water in 24 hours. This paradoxical finding was explained thus: With the onset of rehydration, the concentration of chlorides in the body-fluids fell and there may have been some alteration in the activity of the adrenal cortex. Following these changes, so much less salt presented itself for excretion that (*a*) the kidneys were able to reduce the output of the urine, (*b*) as the concentration of chlorides in the urine also fell, the kidneys were able to raise the concentration of urea in the urine without raising the osmotic pressure.

These experiments confirm that shipwrecked mariners should not drink sea water and cannot possibly do themselves any good by drinking their own urine.

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[1] Black, D. A. K., McCance, R. A. & Young, W. F. (1944) *J. Physiol.* **102,** 406

[1] [*see BMB* **507**]

509

DISCUSSION ON WATER METABOLISM IN SICK AND HEALTHY INFANTS

by W. F. Young, R. A. McCance, F. Avery Jones & R. Dobbs, *Proceedings of the Royal Society of Medicine*, **36**, 219–221, March 1943

Dr. Young pointed out that, because infants frequently suffer from gastro-enteritis, an understanding of their water metabolism, and particularly of the differences in the water metabolism of infants and adults, is of considerable practical importance. A full-term baby contains 75 %–80 % water, and an adult only 60 %–65 %. Further, of the total body-water, 65 % is extracellular in the infant, but only 28 % in the adult. Studies by McCance & Young (1941) have shown that the kidney of the infant at birth is a relatively inefficient organ, and that the infant therefore needs a relatively high intake of water. In premature infants the renal inefficiency is even greater, and the clinically familiar liability of premature infants to become œdematous can be attributed to their low mineral clearances. Glomerular filtration rates are also low as judged by adult standards.

Whenever the output of water by other routes (skin, lungs, bowel) is increased, the infant's urine volume should be maintained by increasing the intake of water. Salt should also be given when there is diarrhœa or vomiting. As the gastro-intestinal secretions may be regarded as isotonic, salt-loss may be replaced by giving an equivalent volume of 0·9 % NaCl. However, a hypotonic solution of salt should always be given to an infant with gastro-enteritis, as some water (but not salt) is being excreted by the skin and lungs. In giving fluids by the intravenous route, solutions of (i) 0·9 % NaCl and (ii) 5 % glucose should be given in varying proportions, according to the estimated requirements.

To enable the body to restore mineral equilibrium, enough water must be given to establish a free flow of urine, but salt requirements should be carefully assessed, whether the oral or a parenteral route is being employed. The rectal route is useful only for supplementing the oral intake when the infant is vomiting but has no diarrhœa. The subcutaneous route should be used only as a supplement to oral feeding, as the solution given is isotonic (0·9 %) NaCl. The intravenous route is often the only one by which the body-fluids can be restored sufficiently rapidly. Whatever route is adopted, accurate charting of output is essential, and a daily record of the body-weight is helpful, in assessing requirements. Change in weight is a more sensitive indication of water balance than serum chemistry.

Dr. McCance emphasized the difference between true dehydration (due to deficiency of body-water) and salt deficiency (usually due to loss of water and salt with partial or total replacement of water), and illustrated his point by a description of personal experiments. By making himself salt-deficient he lost not more than 6 pounds [about 2·7 kg.] weight, and there was clear evidence of severe hæmoconcentration, with a serum-sodium below the normal level. When he dehydrated himself, he lost almost 10 pounds [about 4·5 kg.] without difficulty, his serum-sodium rose to abnormal levels, and there was no reduction in blood volume. True dehydration is seldom encountered in temperate climates, and clinically there is usually a loss of salt as well as water (as in vomiting, diarrhœa, diabetic coma, Addison's disease). Patients should be given saline solutions until the body weight has been restored, and it is then better to use 5 % glucose solution for intravenous infusion unless salt is still being lost. This provides water for lung- and skin-losses without overburdening the kidneys with salt.

Dr. Jones said that it had been shown that the routine use of normal saline infusions after surgical operations involved a risk of water retention with hydræmia and a tendency to œdema of the lungs or of the intestinal suture line. Many sick infants were given the excessive amount of 12 g. of salt, which was equal to the total salt content of an infant's body.

Dr. Dobbs said that practising pædiatricians were not sufficiently informed on the important physiological changes underlying the clinical conditions of " dehydration," " shock " and " collapse " in infants with acute gastro-enteritis. Discussing different routes of administration, he regarded subcutaneous infusion as the safest. The oral route might be inefficient and the indiscriminate employment of the intravenous route might result in such complications as heart failure and cerebral or pulmonary œdema.

REFERENCE

McCance, R. A. & Young, W. F. (1941) *J. Physiol.* **99**, 265

510

THE RENAL FUNCTION OF NEWBORN INFANTS

by H. Heller, *Journal of Physiology*, **102**, 429–440, April 1944

Dr. Heller has worked for a number of years on the comparative physiology of the anti-diuretic principle of the pituitary gland and the investigation reported in this paper follows the work of McCance & Young (1941) on urinary excretion in infancy. The present author has studied the effect of this hormone upon the new-born infant. The action of posterior pituitary extracts on adults is too well known to require description, and small doses have been used for many years in the treatment of diabetes insipidus. Their action is always to reduce the flow of urine even after copious draughts of water. Dr. Heller administered injections of an extract of posterior pituitary gland to infants and studied the effect upon the concentration of the urine and the depression of its freezing point. A dose of 125 milli-units per metre^2 of body surface produced a striking effect upon an adult, but a negligible effect upon an infant 5 days old, and raising the dose to 250 milli-units per metre^2 had little more effect. The author concludes that at birth, and for some time after it, the renal tubules of newborn children are relatively insensitive to the action of the posterior pituitary principle. This is an interesting contribution to the physiology of infancy.

Dr. Heller goes on to show that infants with a reduced intake of water can produce a urine more concentrated than the blood, so that they are not quite in the position of the patient with diabetes insipidus. Nevertheless, even these urines are much less concentrated than those normally passed during adult life, and it is evident that infants must have an abundant supply of water if they are to maintain their normal renal function.

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Mineral Metabolism *

511

SEASONAL AND ANNUAL CHANGES IN THE CALCIUM METABOLISM OF MAN

by R. A. McCance & E. M. Widdowson, *Journal of Physiology*, **102**, 42–49, June 1943

The authors, writing from the Department of Medicine of the University of Cambridge, point out that the capacity of an individual to absorb calcium has been known to vary for a number of reasons. It is believed to be increased during the waves of growth in children; and as rickets, tetany and osteomalacia have been noted to be at their worst in late spring, responsibility has been attributed to the decreased amount of vitamin D formed in the skin in the winter months, because of the effect of vitamin D on calcium absorption.

During experiments on the effect of brown bread on calcium absorption, it was found that the subjects (three human beings for the whole period, five others for a portion of the time) absorbed calcium less freely in November than they did in July. By the following March, in three of the subjects who were still carrying on the experiment, it was clear that the Ca metabolism had changed very much since they had first come under observation.

Experimental methods: The method of estimating absorption of Ca has been described by the authors elsewhere (McCance & Widdowson, 1942a, 1942b). The amount of flour used in the diet (either white or brown) always contributed 40 %–50 % of each person's calories, the rest of the diet was freely chosen but was very similar from one experiment to the next. Most of the figures in the present paper are based on the results of 3-week experiments; some are based on 2-week experiments and a very few on those which lasted only 8 days.

Results: In one man and two women, well defined seasonal changes in calcium absorption were observed. The subjects

* [*see also BMB* **521, 526, 536**]

were under observation for two summers and three winters. They absorbed Ca much more freely in July and August than they did in February and March. This seasonal effect was apparent irrespective of whether white or brown bread was eaten, but the figures were always lower on the latter. The magnesium absorptions were not affected by the seasons and showed a constancy from one season to another which was quite remarkable. It was also found that the three subjects absorbed Ca better in the summers of 1940 and 1942 than they did in 1941. Again there was no variation in the Mg absorption and no seasonal or annual change in the absorption of phosphorus could be observed.

At first it was thought that these changes in absorption were due to changes in the amount of vitamin D in the food or in the amount formed by the sun in the skin. However, the administration of 2,000 international units of vitamin D per day for seven preliminary and 21 experimental days, resulted in negligible increases in the calcium absorption. These fluctuations in Ca absorption appear to be due to some factor or factors in Ca metabolism hitherto undescribed, and the authors suggest that they may be due to variable resistance of the tissues of the body to the action of vitamin D.

From a purely practical point of view it should be noted that all those investigating Ca metabolism over a period of months should be aware of the possibility of having their results vitiated by these variable absorptions.

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[1] [*see BMB* **15**]

512

THE EFFECT OF NATIONAL WHEATMEAL ON THE ABSORPTION OF CALCIUM

by H. A. Krebs & K. Mellanby, *Biochemical Journal*, **37**, 466–468, 1943

This report from the Department of Biochemistry in the University of Sheffield and the *Sorby* Research Institute, Sheffield, is a continuation of the work of McCance & Widdowson (1942), but more particularly in regard to the *National Wheatmeal* flour of 85 %-extraction which has been introduced by the Ministry of Food. Six male volunteers whose ages were between 23 and 31 years were kept on a diet yielding 4100 calories per diem, which included at least 500 g. flour (dry weight) and not more than an average of 0·55 g. calcium per diem. Of the flour, an average of 70 g. (dry weight) was taken in the form of cooked dishes, the remainder as bread. After preliminary trials the main experiment consisted of two periods. During the first, lasting four weeks August-September 1941, *National Wheatmeal* flour (85 %-extraction) was used. The second period of four weeks September-November (1941) was the control period, when "white" flour (75 %-extraction) was used. The phytic-acid phosphorus content of the *National Wheatmeal* flour was 140 mg. per 100 g. and of the bread 41·4 mg. per 100 g.

Under the experimental conditions outlined above, the consumption of *National Wheatmeal* flour was accompanied by an average calcium loss of 0·110 g. per diem. When this flour was replaced by 75 %-extraction flour, the average loss was 0·023 g. per diem. The effect of the *National Wheatmeal* flour was definite in every individual case. In a preliminary experiment where the average intake of *National Wheatmeal* bread was about one pound [about 0·45 kg.] per diem (instead of 1·7 pounds) and the average daily calcium supply 0·94 g., the calcium balance was in equilibrium.

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[1] [*see BMB* **15**]

513

THE RELATIVE EFFICACY OF CALCIUM CARBONATE AND CALCIUM PHOSPHATE IN PREVENTING RICKETS IN RATS

by J. Yudkin, *Biochemical Journal*, **37**, 543–546, 1943

In the absence of vitamin D, calcification of bone is to a large extent dependent on the Ca/P ratio in the diet. With the proposal to add calcium carbonate to *National Wheatmeal* flour, the author, who holds a Sir Halley Stewart Research Fellowship at the *Dunn* Nutritional Laboratory of the University of Cambridge, has compared the power of the carbonate and the phosphate to prevent rickets in rats.

Young rats weighing about 50 g. and kept in separate cages were placed on diets of 85 %-extraction flour plus 1 % sodium chloride to which were added varying amounts of calcium carbonate as *creta preparata* (B.P.) or calcium *mono*hydrogen phosphate ($CaHPO_4$). As controls, the flour was replaced by a white (75 %-extraction) flour, plus daily supplements of aneurin and riboflavin, or a diet containing 76 parts maize meal and 20 parts gluten. The highest amount of each salt added to the diet was equivalent to 3 % of calcium. After five weeks on these diets, the ash content of both femurs of each animal was determined and the knee-joints of the rats were *x*-rayed.

Using white (70 %-extraction) flour and no added calcium, the ash content was between 32 % and 35 %. On adding calcium phosphate, the maximal ash content of about 50 % was reached with approximately 0·4 % calcium, and increasing the calcium to 3 % had little effect. With the carbonate, a maximum ash content of 42 % was reached with 0·2 % calcium. Increasing the calcium carbonate reduced the ash content until at a level of 3 % calcium intake the ash content was only 28 %. Similar results were obtained when *National Wheatmeal* (85 % extraction) flour was used, except that the maximal ash content reached with approximately 0·2 % calcium administered as carbonate was the same as that when phosphate was fed. Similar results were obtained in experiments with maize-gluten diet. The rate of growth on the flour diets was less with the carbonate than with the phosphate; this was reversed on the maize-gluten diet.

The Ca/P ratio in white flour is 0·16, in *National Wheatmeal* flour 0·10, and in maize-gluten 0·10. Addition of calcium hydrogen phosphate (with 1·2 % calcium as phosphate) increases the ratio to approximately 1·0 for all diets. Addition of calcium carbonate increases the ratio to much higher levels; 0·2 % calcium as carbonate gives a ratio of 2·05 for white flour, 1·10 for *National Wheatmeal* flour and maize-gluten. At higher levels of carbonate a rapid rise in the ratio occurs; at 1·2 % Ca the ratio is between 5 and 10 and at 3 % Ca between 13 and 28. Thus maximum calcification recurred in all instances with Ca/P ratio between 1 and 2.

514

EXPERIMENTS ON THE PRACTICABILITY OF INCREASING CALCIUM ABSORPTION WITH PROTEIN DERIVATIVES

by T. C. Hall & H. Lehmann, *Biochemical Journal*, **38**, 117–119, 1944

McCance, Widdowson & Lehmann (1942) showed that by increasing the "protein" intake from about 60 to 160 g. protein per diem, the calcium absorption was increased. The present authors have examined the urinary excretion of calcium following the ingestion with peptone or with lactose. They have devised a powder containing 100 mg. calcium hydrogen phosphate ($CaHPO_4$), 2,000 mg. peptone, 200 mg. glutamic acid, 550 mg. lactose and 150 mg. gum acacia. A powder in which lactose replaced peptone and glutamic acid was used as a control.

After 14 hours, during which food and water were withheld, 12 control powders were given with 750 ml. water. The urine was collected during the next 5 hours and its calcium content was determined after conversion to ash. After an interval of at least 3 days, but not more than 5 days, 12 "peptone" powders were given and the procedure was repeated.

Nineteen experiments were carried out on 18 inmates of a mental hospital. In 14 subjects the urinary calcium was higher after the "peptone" powders than after the "control" powders. Even when the amount of peptone was halved, a rise in the urinary calcium was still evident. In agreement with the work of McCance & Widdowson (1939), where a normal serum-calcium existed before the test, the ingestion of the "peptone" powders did not raise the serum-calcium level. In one subject, however, with an initial serum-calcium of 0·8 mg. per 100 ml., the serum-calcium rose to the normal level on the "peptone" powder but not on the "half-peptone" or lactose powders. After withdrawal of the

" peptone " powder, the serum-calcium declined to its initial level in 3 weeks.

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[1] [*see BMB* **99**]

515

PHYTIC ACID AND IRON ABSORPTION

by R. A. McCance, C. N. Edgecombe & E. M. Widdowson, *Lancet*, **2**, 126–128, 31/7/43

An element must presumably be in solution if it is to be absorbed, and the presence in the diet of a radicle which precipitates the element will depress absorption. It has been shown by McCance & Widdowson (1942 a, b) that phytates depress calcium absorption. Barrett (1942) has shown that calcium depresses oxalate absorption by the formation in the intestine of the relatively insoluble calcium oxalate. In experiments on the effect of brown bread on absorption of calcium, Widdowson & McCance (1942) found some evidence that the phytic acid in brown bread might interfere with the absorption of iron.

Four normal men and 5 normal women were used for the present experiments. Each person carried out 4 tests, 2 to measure the effect of sodium phytate on the absorption of ferrous iron and 2 to make a similar study with ferric iron. In some cases additional experiments were carried out in which phosphates replaced phytates. After an overnight fast the subject came to the laboratory and a sample of blood was taken. Breakfast consisted of bread and jam and ferrous or ferric ammonium sulphate. The amounts eaten by each individual were equal to 1·8 g. bread, 1·1 g. jam and 8 mg. iron per kg. body weight. The iron salt was mixed with jam and spread on the bread. Sodium phytate was incorporated into the diet when desired by mixing it with the bread dough (made from low-extraction flour) before baking. When the effect of phosphate was being studied, di-sodium hydrogen phosphate was added to the bread in similar proportion. Samples of blood (from the ante-cubital vein, using stainless steel needles) were collected 1½–2 hours, 4–5 hours, and 7–8 hours after breakfast. In the estimations, the iron of the serum was converted to ferric thiocyanate, and the intensity of the colour developed was compared with that of a standard in a photo-electric colorimeter.

It was found that sodium phytate interfered with the absorption of iron. Disodium hydrogen phosphate had a similar but less regular effect. The absorptions of both ferrous and ferric iron were affected by sodium phytate, but there was a greater retarding effect on the absorption of the latter.

Without the sodium phytate it was found that appreciable quantities of iron were absorbed from the intestine after a large dose of soluble iron salt. If sodium phytate added to bread has such an effect, it is fairly certain that the phytates of whole cereals would behave similarly and would be capable of immobilizing iron present in the rest of the diet.

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[1] [*see BMB* **194**] [2] [*see BMB* **15**] [3] [*see BMB* **14**]

*Vitamin-B Complex * : Nitrogen*

516

STUDIES IN VITAMIN B DEFICIENCY WITH SPECIAL REFERENCE TO MENTAL AND ORAL MANIFESTATIONS

by A. G. Clarke & F. Prescott, *British Medical Journal*, **2**, 503–505, 23/10/43

This report from the *West End Hospital for Nervous Diseases* and the *Wellcome Research Institute*, London, deals with 17 cases of deficiency of the vitamin-B complex in patients who were being treated primarily for functional nervous disorders, the main symptoms of which were depression, headache, insomnia, and loss of appetite. Other symptoms were lack of energy, fatigue on slight exertion, breathlessness, inability to concentrate, and nervous dyspepsia. There was one case of psychosis and four patients had peripheral neuritis. All the patients had glossitis and nearly all cheilosis. Seven typical case records are given in the original paper, which also contains photographs illustrating the effect of treatment. Although in most cases the deficiency seems to have been determined by the wartime diet, it was actually precipitated only when mental or physical factors led to loss of appetite or impaired absorption. These factors included adherence to special diets (for peptic ulcer, obesity, and hypertension) and conditions interfering with the absorption of food (vomiting and gastro-intestinal diseases).

Striking improvement occurred in all the cases as the result of treatment with vitamins of the B complex without alteration of the environment or special psychological measures.

* [*see also BMB* **493, 494, 495, 496, 520, 522, 523, 524, 533, 534, 536, 537, 539, 543**]

517

SOME PROBLEMS IN RIBOFLAVIN AND ALLIED DEFICIENCIES

by H. S. Stannus, *British Medical Journal*, **2**, 103–105 & 140–144, 22/7/44 & 29/7/44

These two papers are the substance of the Lumleian Lectures delivered before the *Royal College of Physicians* of London in April 1944. During the years 1912 to 1913, the author published an account of an outbreak of pellagra amongst long-term prisoners in Nyasaland whose staple diet was rice. Attention was directed to a group of symptoms which tended to precede the onset of the classical signs of pellagra by some months, and in some instances persisted without the development of frank pellagra. The manifestations included : soreness of tongue and lips with a characteristic lesion at the corners of the mouth, and with a similar condition about the nostrils, palpebral fissures, the free border of the prepuce, the vulva and anus, together with a dermatosis of the scrotum, often also involving the skin of the adjacent part of the thigh. This syndrome, limited to lesions of the oral aperture, was not uncommon among the general native population. Amongst the prison patients, deafness and dimness of vision were also noted but not associated at that time with the syndrome.

In 1936, the author collated a number of published observations which he considered were akin to the original syndrome and used the term " pellagra-like conditions " in order to focus attention upon malnutrition as the cause. Moore (1930–1937) and Landor & Pallister (1935) published accounts in which the original syndrome was described and attributed to a dietetic deficiency which responded to autoclaved yeast or marmite.

In 1938, Sebrell & Butler extended the animal experiments of Goldberger & Tanner (1925) to include man. Eighteen women were kept on a riboflavin-free diet, and after 94 to 130 days, 10 of them developed lesions on the lips, at the angles of the mouth, in the vestibule of the nose, etc., comparable to those described by Goldberger & Tanner as " pellagra sine pellagra " in their animals and those described by Stannus in man. As these lesions disappeared after riboflavin therapy they suggested that " the term ariboflavinosis be added to the nomenclature of the vitamin-deficiency diseases as a designation for the clinical condition due to riboflavin deficiency." Stannus prefers the term " hypo-riboflavinosis ", as there is reason to believe that death occurs long before the advent of ariboflavinosis. Later work has extended the symptoms and signs of ariboflavinosis (*see* Sydenstricker, 1941), but the present author holds that the differences between the newer syndrome of ariboflavinosis and those included in the older syndrome are differences in degree only. Many groups of symptoms, combined into a series of overlapping syndromes due to malnutrition during the Spanish Civil War 1936–9 have been described by Márquez Blasco & Peraita (1940), Jiménez García & Grande Covián (1940).

As riboflavin forms the link between the anærobic dehydrogenase system and the ærobic cytochrome : cytochrome-oxidase : oxygen system, Stannus suggests that in

hyporiboflavinosis the capillary endothelium will be one of the first tissues to suffer from the induced anoxia. This anoxia produces a derangement of function which he terms "capillary dysergia", with the development of loss of tone, dilatation and a decreased rate of flow of the blood, which in turn leads to a disturbance of the internal environment and so to a metabolic disorder of the adjacent tissues. If this is so, the signs of hyporiboflavinosis will be first manifested in those tissues with a high metabolic activity and a marked degree of capillarity. On this hypothesis the author offers an explanation of the nature and distribution of the signs and symptoms of a riboflavin deficiency. The worsening of the hyporiboflavinosis lesions as a result of the administration of nicotinic acid is attributed to the vasodilator action of that substance. Of the ocular manifestations, Stannus holds that dimness of vision, which has to a large extent been disregarded by American observers, is part of the general capillary dysergia which produces a condition akin to a retrobulbar optic neuropathy. As to the question of corneal vascularization, he deplores the fact that few workers in this field appear to be cognisant of the work of Graves (1934) on the vascularization of the cornea and believes that the vascular changes can be successfully studied only with the aid of capillario-dilator and -constrictor drugs. The author does not believe that it is possible to formulate any satisfactory notation for describing the degree of vascularity of the limbus and cornea, as has been attempted by Lyle, Macrae & Gardiner (1944). From his observations by slit-lamp microscopy of the eyes of nearly 5,000 subjects, between 3 and 93 years of age, Stannus believes that there is always a complete limbic plexus and that there is no narrow avascular zone between the limbic plexus and the sclero-corneal junction. Whilst corneal vascularization is not an unequivocal sign of hyporiboflavinosis, it does occur.

Following the work of Philpot & Pirie (1943), who suggested that the corneal supply of riboflavin might be through the lacrimal secretions rather than the blood of the limbal loops, Stannus believes that the earliest trophic changes in the cornea, due to riboflavin deficiency following a decreased lacrimal secretion, produce proliferation-promoting intercellular hormones (Loofbourow, 1942 ; Loofbourow, Webb, Loofbourow & Abramowitz, 1942) which stimulate the loops of the limbic plexus to throw out new vessels. This would also afford an explanation of the similar if not identical corneal vascularization met with in vitamin-A-deficient rats (Wolbach & Howe, 1925). The neurological symptoms included in the syndrome under discussion, all of which are sensory and comprise muscle weakness, inco-ordination, ataxia, paræsthesia and loss of visual and auditory acuity, are considered to arise from disturbances of the capillary supply, particularly to synaptic structures in the cerebellum, and are in accord with the degree of capillarity of the various parts of the nervous system as determined by Craigie (1940). The author believes that the little pathological evidence which is available concerning hyporiboflavinosis supports his viewpoint.

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[1] [*see BMB* **523**]

518

THE NUTRITIVE VALUE OF THE NITROGENOUS SUBSTANCES IN THE POTATO AS MEASURED BY THEIR CAPACITY TO SUPPORT GROWTH IN YOUNG RATS

by H. Chick & M. E. M. Cutting, *Lancet,* **2,** 667–669, 27/11/43

This paper from the Nutrition Division of the *Lister* Institute was the result of work carried out to test the advisability of substituting potatoes for a proportion of the wheat consumed in Britain, with a view to economy of shipping space. Dried potatoes differ little from flour from a calorific point of view, but, as usually eaten, the potato has a much lower calorie value as it contains 75 % of water, as opposed to 33 % in the case of bread. Potatoes are a better source of vitamins than wheat. They have approximately as much vitamin B_1, riboflavin and nicotinic acid as wheat and, in addition, may contain appreciable amounts of vitamin C. No fat-soluble vitamins are present.

The potato contains about half the amount of nitrogen present in wheat and about ¼ to ⅓ the amount of protein. The potato differs from wheat in having about half its nitrogenous substances in the form of amides, amino-acids and purines. Kon & Klein (1928) have demonstrated that, weight for weight, the nitrogen of the potato is at least as efficient as the nitrogen of wheat for the maintenance of nitrogenous equilibrium in adult man and animals. Unfortunately, however, the large proportion of starch in potatoes suggests that it would be necessary to eat an impossible bulk in order to obtain enough nitrogenous matter for the optimum requirements of growth. The experiments reported in the present paper had the object of comparing the effect of potatoes with wheat on the growth of young rats from the time of weaning.

King Edward potatoes were used, containing 1·8–2 % of nitrogen (reckoned on the dry weight). In some experiments the protein separated from the expressed juice was used as a source of nitrogen, in others the protein-free juice was used, and in others the whole potato. Vitamin and mineral supplements were given as well. The intake of dried food for all rats in one group was measured together, as well as the dry weight of the fæces passed and their nitrogen content.

The value of the nitrogenous substances in the potato for supporting growth in young rats was found to be somewhat greater than those of whole wheat. Addition of sodium citrate to the wheat diet in amounts sufficient to make the alkalinity of its ash equal to that of the potato was without effect. A diet containing 9 % of tuberin, the soluble protein of potato juice, was equal to one containing 11 % whole wheat protein, but not equal to one containing 11 % casein.

The results obtained can be explained only by assuming that some portion of the non-protein nitrogenous substances in the potato complements the amino-acids of its protein to produce a mixture of biological value not less than that of protein itself.

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519

THE BIOLOGICAL VALUE OF MIXED PROTEINS IN FOOD SERVED IN ROYAL AIR FORCE AIRMEN'S MESSES

by T. F. Macrae, K. M. Henry & S. K. Kon, *Biochemical Journal,* **37,** 225–230, July 1943

Relatively little is known of the biological value of proteins as supplied in mixed human dietaries. The authors of this paper report experiments to determine the biological value of the combined proteins in food served at 4 Royal Air Force Stations. At each Station the total food available to 5 airmen at all meals during 1 week was collected, stored at — 20° C. for 7 days, and pulverized in the frozen state. The pulverized frozen material was dried at a temperature of 70° C., and then minced and finally pulverized in a coffee-mill. The resultant product was a fine brown-coloured powder.

The biological values and 'true digestibilities' of this product as obtained from each of the 4 Stations were determined by the method of Mitchell (Mitchell, 1924 ; Mitchell & Carman, 1926), using rats. The results of the tests are tabulated in full. They showed that the product obtained from the diets from each of the 4 Stations had high biological

values (from 77·6 to 81·2) and a high degree of digestibility, 'true digestibility' coefficients ranging from 83·5 to 86·3). Of single foodstuffs, only eggs and milk surpassed the mixed proteins of these diets in biological value. In their discussion of results, the authors compare the figures obtained with those from comparable experiments from India and China.

This paper is briefly reported here because of the interest of the method of converting a mixed diet to a homogeneous and stable product of great convenience for experimental purposes. For full details of method and results, reference should be made to the original paper.

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Vitamins and Bone-Growth

520

EFFECT OF VITAMIN B ON THE GROWTH OF FIBROBLASTS

by E. Paterson & M. V. Thompson, *Biochemical Journal*, **37,** 501–505, October 1943

The effect on growth of the B-complex vitamins has usually been examined *in vivo* and vitamin B_1 has been assayed by its effect on the growth of laboratory animals. These experiments from the *Southport* Research Laboratories of the *Christie* Hospital and *Holt* Radium Institute, Manchester, were designed to test whether the growth-promoting effect could be demonstrated *in vitro* on explants of fibroblasts from the choroidal and sclerotic layers of the eyes of 9-day chick embryos cultured by the hanging-drop method. Under standard conditions of preparation and culture, growth was assessed at the end of 48 and 96 hours in terms of the increase in area of the culture determined by measuring with a planimeter the area of a projected drawing of the explant. All the results were compared statistically, the degrees of freedom ranging between 33 and 87.

It was found, in the first place, that extracts prepared from the brains of pigeons, in which beri-beri had been produced by the method recommended by Kinnersley, Peters & Reader (1928), were growth-promoting to fibroblasts, the effect being proportional to the concentration of the extract. It was then shown that such extracts were significantly less growth-promoting than extracts from normal pigeon brains. Further experiments involving the separate addition of aneurin and an extract of brewer's yeast to growing explants gave rise to the conclusion that some component of the B complex is growth-promoting *in vitro*. This factor is probably not either aneurin or biotin. The addition of aneurin could not be shown to increase growth whether the medium was or was not deficient in its content of the B-complex vitamins.

The methods described in this paper may be satisfactory for further direct testing of the growth-promoting properties of other constituents of the B complex.

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521

SOME EXPERIMENTS ON THE POSSIBLE RELATIONSHIP BETWEEN VITAMIN C AND CALCIFICATION

by G. Bourne, *Journal of Physiology*, **102,** 319–328, December 1943

It seems well established that adequate amounts of vitamin C are essential for the laying down of the organic matrix of bone (see, for example, Bourne, 1942a), but the evidence so far available favours the view that the vitamin plays no part in the process of calcification.

The work reported in this paper from the University Laboratory of Physiology, Oxford, seeks to clear up this point. Guinea-pigs, maintained on a scorbutic diet and injected with graded supplements of vitamin C, were fed alizarin Bordeaux (1 : 2 : 5 : 8 tetrahydroxyanthraquinone), the special property of which is to stain newly-deposited bone salt. The results suggested that the vitamin does play a part in the laying down of bone salt. These experiments, however, did not show how far the better deposition of bone salt in the animals receiving supplements of the vitamin was simply due to greater availability of the necessary matrix.

By a technique previously described (Bourne, 1942b), observations were made on the calcification process in a 1 mm. hole bored aseptically in the femur of each of a number of guinea-pigs receiving ascorbic acid at various levels. By an ingenious device an attempt was made to differentiate the effects of ascorbic acid on developing trabeculæ and (if any) on calcification. These experiments were followed by observations on costo-chondral junctions stained variously by von Kossa's silver nitrate method for demonstrating bone salt and by cobalt chloride and ammonium sulphide (Gomori) for freshly deposited calcium phosphate. The results again suggested that vitamin C is associated with the process of calcification, but it was found impossible to separate its functions in respect of bone-matrix formation and calcification since these two processes are simultaneous.

Further investigations supported the results of Shwachman & Gould (1942) that bone phosphatase is reduced in the scorbutic guinea-pig. "It appears likely that one of the functions of vitamin C is to allow the production of a phosphatase-impregnated bone matrix upon which bone salt is immediately deposited. Vitamin C may play some part in the formation or stabilization of alkaline phosphatase."

Neither vitamin P ("citrin" Roche) nor sodium citrate resulted in the formation of more osteoid tissue, or in the deposition of more bone salt, than vitamin C alone.

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[1] [*see BMB* **17**]

Ocular Manifestations of Nutritional Deficiency *

522

OCULAR SIGNS OF RIBOFLAVIN DEFICIENCY

by W. J. W. Ferguson, *Lancet*, **1,** 431–433, 1/4/44

Since Sydenstricker, Sebrell, Cleckley & Kruse (1940) described certain ocular signs and symptoms which responded to riboflavin therapy, many workers have reported widely conflicting results. The author of this paper, who is the senior ophthalmic surgeon at the *Sheffield Royal Infirmary*, believes that differences have arisen mainly through lack of experience in the use of the slit-lamp, an imperfect understanding of the normal variations of the appearance and vascularity of the limbus, and the inclusion of corneal vascularization due to other causes than riboflavin deficiency; but a particular recognizable type of corneal vascularization which rapidly responds to adequate riboflavin therapy does exist. He deplores the term "circumcorneal injection," as the limbic plexus becomes congested and engorged on the slightest provocation.

Riboflavin deficiency produces a vascularization which is an extension of the normal limbic vascular system to the true cornea. The vessels are of very small calibre and run superficially immediately under the epithelium, and they are best seen with a corneal microscope and slit-lamp when observed by light reflected from the iris. In reflected light, the vessels appear as very fine cobweb-like lines, in which an irregular spasmodic movement of the blood-corpuscles can be seen. Such a vascularization "should be visible *all round the corneal circumference* and not in isolated patches, though it need not be present to the same depth in both corneæ simultaneously." Here the author is in agreement with Gregory (1943) and Pirie (1943).

* [*see also BMB* **517**]

No sign of a deeper corneal vascularization nor any evidence of the consequent development of corneal inflammation or opacities has been encountered. Visual fatigue, but no loss of visual acuity, often accompanies this corneal vascularization. Fissures at the angles of the mouth, a dry scaly skin, a history of dry lips with a tendency to crack and redness of the tip of the tongue, occur in a large proportion of these cases. Riboflavin in adequate dosage is followed by a rapid improvement in the subjective symptoms and a complete cessation of the circulation in the abnormal corneal vessels in 2–3 weeks.

The vascularity of interstitial keratitis is deeper, with larger vessels having the broom-head type of distribution; that following superficial corneal ulceration or inflammation is coarser, localized and easily visible with a binocular loupe.

An abnormal corneal vascularization characteristic of a very mild riboflavin deficiency was found in 7·8% of 422 persons (industrial workers, students, institutional inmates and out-patients). In 13 subjects in which the effect of 10 mg. daily of riboflavin was followed over an adequate period (3–4 weeks) a cessation of the abnormal corneal circulation was observed.

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CORNEAL VASCULARISATION IN NUTRITIONAL DEFICIENCY

by T. K. Lyle, T. F. Macrae & P. A. Gardiner, *Lancet*, **1**, 393–395, 25/3/44

This paper reports work carried out by three officers of the *Royal Air Force* on the instructions of the Director-General of Medical Services and the Director of Hygiene, Air Ministry. The relationship between corneal vascularization and nutritional status in nearly 4,000 *Royal Air Force* personnel has been studied for over 2 years at 22 localities, 10 in Great Britain, 12 overseas. The overseas stations varied from subarctic to tropical desert. At selected stations the effects of supplementary diet were studied.

The nasal, inferior and temporal quadrants of both eyes were examined and four types of vascularity were recorded.* Type A (score 0) a few small vascular twigs only on the limbus; Type B (score 1) increased vascularity of limbus; Type C (score 2) as in Type B but with vascular twigs in clear cornea; Type D (score 3) with vascular loops in a clear cornea.

In Britain, where military rations were considered nutritionally satisfactory, 2,016 subjects were examined throughout the year, the average score was 5·1, and 22% had a score greater than 6. In overseas stations, where the standard of feeding was excellent, the average score was 4·4, with 17% scoring above 6. At a tropical station, where the diet consisted of tinned fruit and vegetables, some poor quality fresh meat, much tinned meat, no fresh fish or eggs and where peanuts and ascorbic acid were issued, the average score was 8·7 with 75% having a score of more than 6.

The effect of various supplements was examined at three stations in Britain, and on two occasions at a sub-arctic station overseas where the findings obtained initially corresponded closely with those in Britain.

i. Unselected subjects in civilian quarters, and consequently receiving rations similar to those of civilians, were divided into four groups. After 21 days, a significant improvement was detectable in those receiving 4 mg. riboflavin daily, with or without 100 mg. ascorbic acid. Vitamin A, 50,000 international units daily, had no significant effect. This experiment was made early in 1942.

ii. At a sub-arctic station in the summer of 1942, neither riboflavin 10 mg. daily nor nicotinamide 100 mg. daily produced a significant improvement in the groups taken as a whole, but some subjects with the most definite corneal vascularity did improve, especially on the riboflavin supplements.

iii. At a station near London in spring 1943, 353 airmen who had been on military rations for some months were divided into three groups: (*a*) a control group, (*b*) a second group which received 10 mg. riboflavin daily, (*c*) a third group which received 3 mg. aneurin, 3 mg. riboflavin, 50 mg. nicotinamide, 10 mg. calcium-*d*-pentothenate and 3 mg. pyridoxin. No significant difference was observed in the three groups after 28 days.

iv. In the summer of 1942, 289 airmen at the sub-arctic station were examined and their diet, which had not been entirely satisfactory, was supplemented with a mixture of highly nutritious foodstuffs which were calculated to add about 4,000 international units vitamin A, 0·5 mg. aneurin, 0·8 mg. riboflavin, 4·5 mg. nicotinamide and 120 mg. ascorbic acid to the daily ration. Re-examination after 5 weeks showed an improvement in the group as a whole, especially in those with initially high degrees of vascularity.

v. The effect of supplements of liver and kidney to the diet, calculated to about 1·5 mg. riboflavin daily, was followed in 82 airmen for 4 weeks and 50 airmen for 10 weeks in the spring of 1943. No significant improvement was observed.

The authors consider that, although many subjects on excellent dietaries had blood-vessels on the cornea, the average degree of corneal vascularity in a group of subjects is a reliable index of their general state of nutrition. Riboflavin is not the only nutrient concerned in the prevention of corneal vascularization; it appears that other factors present in fruits and vegetables are operative.

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AMBLYOPIA DUE TO A VITAMIN DEFICIENCY

by P. B. Wilkinson & A. King, *Lancet*, **1**, 528–531, 22/4/44

This paper is based on observations made by the authors in Hong-Kong during the latter half of 1940. During this period 15 patients were seen who complained of gradual or sudden onset of dimness of vision. This symptom was associated with soreness of the tongue, giddiness, paræsthesiæ, weakness of the limbs and other manifestations believed to be of nutritional origin. There were no external ocular signs of nutritional deficiency (although it was not possible to make slit-lamp examinations). Temporal pallor of the optic discs was seen in 3 patients, and in a fourth patient who was an undoubted pellagrin there was a left-sided optic atrophy which later became bilateral. In the remaining 11 patients, no abnormality of the optic discs was seen, but in these cases the visual defect had been of shorter duration. In 13 cases the pupillary reactions to light were retarded. In all but 2 cases perimetric examination showed constriction of the visual field. Radiological, neurological and hæmatological findings were not such as to account for the visual defects.

In discussing the ætiology of the condition, the authors draw attention to a deterioration which had taken place in the diet of the social groups from which the patients came. Gradual improvement followed administration of dried yeast in doses of approximately 4 g. 3 times daily. Much more rapid improvement was obtained by nicotinic acid in doses of 100 mg. daily by mouth.

The authors comment that it has for some time been recognized that malnutrition may cause amblyopia, and that their experience in this small series of cases supports this view.

Disease of Teeth and Buccal Cavity

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MOTTLED ENAMEL, M-HYPOPLASIA AND DENTAL CARIES

by J. D. King, *Dental Record*, **64**, 102–110, May 1944

A field investigation was made of the incidence and extent of enamel mottling, M-hypoplasia and dental caries in elementary schoolchildren living in parts of Essex (high fluorine water), Oxfordshire (fairly high fluorine water) and Suffolk

* [*see BMB* **517** for a criticism of the notation used.]

(low fluorine water). The districts were selected in consultation with colleagues (Bowes & Murray, 1935; Lowater & Murray, 1937; Murray & Wilson, 1942; Wilson, 1941) who had already reported various related biochemical, clinical and geological studies in these and other parts of Britain. Many workers in different countries had previously observed that the *general incidence level* of dental caries is usually lower in areas where the population is largely affected by mottling of the enamel, and in such districts the drinking water frequently contains a higher fluorine content than elsewhere. The inference has been that the fluorine intake plays some part in depressing caries susceptibility. It was then reasonable to expect that, using the same diagnostic criteria for mottling, similar findings would be obtained if the *individual teeth* were considered in detail, as distinct from a more general assessment of the state of the mouth as a whole. The apparent absence of any definite relationship between caries and mottling in individual teeth disclosed by the present study was therefore of interest. Further, while the data again showed a definite association between caries susceptibility and certain forms of hypoplasia, the author was unable to trace any association whatever between such enamel defects and presence or absence of mottling. The diagnostic criteria for the three dental conditions considered were as follows:

Enamel Mottling:

(*a*) For children in the Maldon and Burnham districts of the county of Essex, mottling was classified as:

No mottling (0).

Slight mottling (1): opaque white patches or streaks involving up to one-half of the occlusal or up to one-third of the buccal surface of the teeth.

Medium mottling (2): similar conditions but involving more than one-half of the occlusal or more than one-third of the buccal surface of the teeth.

Severe mottling (3): brown staining of the occlusal or buccal surface of the teeth, with or without pitting.

(*b*) For children in the Bampton, Hook Norton, Launton and Littlemore districts of the county of Oxfordshire, and Ipswich (Suffolk), enamel mottling was recorded after the methods of Dean (1938) but the degree of mottling was so tabulated as to fall into none, slight, medium and severe grades, in order to conform to tabulation of the data for comparison with extent of caries and hypoplasia.

Dental caries: The method for recording incidence and extent of dental caries was identical with that used by King (1940) the diagnosis being dependent on examination with an illuminated mirror and a probe.

M-hypoplasia: The hypoplasia recorded here excluded the more generally recognized but relatively uncommon form illustrated in most text-books and was confined to the much more prevalent type of defective calcification first described by M. Mellanby (1923, 1927, 1934) and referred to by King (1940) as M-hypoplasia. In estimating the incidence and extent of the latter defect, fully erupted teeth were graded primarily according to the surface roughness of their buccal enamel as tested with a fairly sharp dental probe. As described previously, the grading was then given hypoplasia figures of 0, 1, 2 or 3, the figure 0 indicating "normality" of calcification or no hypoplasia.

Results

i. *Essex*

Of the 50 children of 12–14 years examined, 46 (92%) showed some evidence of enamel mottling according to the writer's classification, and this figure would probably have been higher had the diagnostic criteria of Dean (1938) been employed. In this group of subjects, 18 (36%) were completely free from caries as judged by careful mirror and probe examination. Considering the first permanent molars, and first and second premolars separately and in more detail, the following findings were reported:

First permanent molars: Of the 175 examined, 109 (62%) were mottled, 55 (31%) were carious and 84 (46%) were grade 2–3 (moderately or severely) hypoplastic. As regards the possible association between these three conditions:

of 109 mottled teeth, 26% were carious;
of 66 non-mottled teeth, 41% were carious;
of 109 mottled teeth 46% were grade 2–3 hypoplastic;
of 66 non-mottled teeth, 51% were grade 2–3 hypoplastic;
of 84 grade 2–3 hypoplastic teeth, 65% were carious.
of 91 grade 0–1 hypoplastic teeth, none were carious.

First and second premolars: Of the 361 examined, 264 (73%) were mottled, 11 (3%) were carious and 176 (50%) of 353 teeth * were grade 2–3 hypoplastic. As regards the possible association between these conditions:

of 264 mottled teeth, 2% were carious;
of 97 non-mottled teeth, 5% were carious;
of 256 mottled teeth,* 46% were grade 2–3 hypoplastic;
of 97 non-mottled teeth, 59% were grade 2–3 hypoplastic;
of 176 grade 2–3 hypoplastic teeth, 4% were carious;
of 177 grade 0–1 hypoplastic teeth, none were carious.

ii. *Oxfordshire*

Of the 31 children of 12–14 years examined, 30 (97%) gave evidence of some degree of enamel mottling in one or more teeth per mouth, classified after the method of Dean; 5 (16%) were free from dental caries. Individual details were as follows:

First permanent molars: Of the 117 examined, 81 (69%) were mottled, 65 (56%) were carious and 79 (68%) were grade 2–3 hypoplastic. Regarding the possible association between these three conditions:

of 81 mottled teeth, 58% were carious;
of 36 non-mottled teeth, 50% were carious;
of 81 mottled teeth, 67% were grade 2–3 hypoplastic;
of 36 non-mottled teeth, 69% were grade 2–3 hypoplastic;
of 79 grade 2–3 hypoplastic teeth, 75% were carious;
of 38 grade 0–1 hypoplastic teeth, 16% were carious.

First and second premolars: Of the 211 examined, 143 (68%) were mottled, 12 (6%) were carious and 80 (38%) were grade 2–3 hypoplastic. As to the association between these conditions:

of 143 mottled teeth, 7% were carious;
of 68 non-mottled teeth, 3% were carious;
of 143 mottled teeth, 31% were grade 2–3 hypoplastic;
of 68 non-mottled teeth, 53% were grade 2–3 hypoplastic;
of 80 grade 2–3 hypoplastic teeth, 8% were carious;
of 131 grade 0–1 hypoplastic teeth, 5% were carious.

iii. *Suffolk*

From the 200 children of 12–14 years examined, data for the premolar teeth only were recorded and of the 1,409 teeth studied 554 (39%) were mottled, 190 (13%) were carious and 593 (42%) were grade 2–3 hypoplastic. With regard to any association between these conditions:

of 554 mottled teeth, 22% were carious;
of 855 non-mottled teeth, 8% were carious;
of 554 mottled teeth, 52% were grade 2–3 hypoplastic;
of 855 non-mottled teeth, 36% were grade 2–3 hypoplastic.
of 593 grade 2–3 hypoplastic teeth, 29% were carious;
of 816 grade 0–1 hypoplastic teeth, 2% were carious.

Statistical analysis of the data was then made by the χ^2† and another test, the results of which can be expressed briefly as follows:

(*a*) In no instance could any negative or inverse association be found between enamel-mottling and dental caries. Indeed, in the Essex premolar teeth a positive association existed between the two conditions—the more the mottling, the more the caries.

(*b*) Only in the Oxfordshire premolar teeth was there any negative association between mottling and M-hypoplasia, *i.e.* the more the mottling the less the hypoplasia.

(*c*) A positive association was found between M-hypoplasia and caries in all but the Oxfordshire premolar teeth, and in those the caries incidence was too low for any inferences to be drawn with safety.

(*d*) From (*b*) and (*c*) it is apparent that the only instance

* [Of the 361 premolar teeth examined for mottling and caries, 8 were so severely mottled that insufficient enamel was present on the buccal surface to allow hypoplasia grading.]

† [Particulars of this statistical test are to be found in: Fisher, R. A. (1936) *Statistical methods for research workers*, 6th ed. Edinburgh.]

of a negative association between mottling and either of the other two conditions was that between mottling and M-hypoplasia of the Oxfordshire premolar teeth, in which M-hypoplasia and caries were unrelated.

(*e*) As regards mottling and caries, when analysis was confined to those teeth showing the more severe grades of mottling, there was no significant association between the conditions.

Deciduous dentition

The details relating to the deciduous teeth were not analysed in detail. In Essex, of 50 children under the age of six years, 31 (62 %) showed evidence of enamel mottling in one or more deciduous teeth per mouth, according to the writer's classification. In Oxfordshire, of 39 children between the ages of five and twelve years, 24 (62 %) showed mottling according to Dean's classification. In the latter district, of the 525 deciduous teeth examined 127 (24 %) were affected by some degree of mottling, and of these 62 % were also carious; 141 out of 398 (35 %) non-mottled teeth were carious. A negative association between mottling and caries was again lacking but a positive relationship existed between M-hypoplasia and caries. It was also noted that even in the Essex area, where mottling of the permanent teeth was relatively severe, deciduous mottling was only of minor degree and mainly assumed the form of slight opacity of the tooth cusps, particularly of the posterior teeth.

In a discussion of the findings, it is mentioned that the Essex children's drinking water contained up to 5 parts per million of fluorine: in Oxfordshire there was a varying but generally lesser amount; and in the Suffolk district there was a relatively low fluorine content. Thus, there was confirmation of the results of previous workers as regards a lower *general* caries incidence level in communities whose drinking water contained comparatively large quantities of fluorine. On the other hand, such findings did not hold good when the caries susceptibility of the *individual* teeth was considered. The author points out that this apparent anomaly might be related to the fact that there is no evidence that all enamel opacities (other than those due to undermining caries) are always associated with the fluorine intake. Moreover, it is possible that fluorine may exert a protective influence on the teeth not so much by altering the developmental composition and structure of the teeth as by some unknown effect on the saliva or oral micro-flora; if the latter be true then the existence of mottling *per se* might be merely a coincidental reflection of conditions at the time of tooth development. The author concludes: "At the present time it is perhaps safer to limit our speculations to the statement that, with a given fluorine level, the visible demonstration of enamel mottling may depend on the fluorine intake or on other unknown factors on which, in turn, resistance to caries may be dependent or independent."

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THE IMPROVED DENTITION OF 5-YEAR-OLD LONDON SCHOOL-CHILDREN: A COMPARISON BETWEEN 1943 AND 1929

by M. Mellanby & H. Coumoulos, *British Medical Journal*, 1, 837–840, 24/6/44

A brief description is first given of the experimental and clinical investigations (Mellanby, 1918, 1923) which led up to the demonstration that susceptibility to dental caries was closely associated with the structure of enamel and dentine and with the calcifying properties of the dietary. This work has been set out in detail in a series of special reports, published by the Medical Research Council (Mellanby, 1929, 1930, 1934, 1936) in which it was shown that vitamin D and other calcifying factors had a protective influence on the teeth. It was therefore advised that, in order to improve the dental health of the population, the dietary of pregnant women, infants and children should be changed to one of higher calcifying quality. It was suggested that this alteration could be best effected by (*a*) an increased consumption of milk, eggs and other foods comparatively rich in calcifying factors; (*b*) decreased intake of cereals such as bread, oatmeal and oatmeal preparations; and (*c*) addition to the diet of cod-liver oil or some other source of vitamins D and A, especially the former. During the last 25 years or so, the British Government has made special efforts to improve the nutrition of expectant and lactating mothers and their offspring and amongst the measures introduced were increased supplies of milk (free or at a much reduced price) and, more recently, of cod-liver oil. Such improvements were, in fact, precisely those which Mellanby's investigations had indicated would have a beneficial influence on dental health, and therein lay the reason for the present study. Already, in 1929, a dental examination had been made of 1,300 London children in which the incidence of caries and hypoplasia was recorded (Board of Education, 1931) and in 1943 it became possible to study these conditions in a group of London children of comparable age and living in the same districts; on both occasions the same examination criteria were adopted. The following results show that changes in both surface structure of the teeth and caries incidence have occurred between 1929 and 1943—the structure of the teeth has become better and the amount of caries has been reduced.

Table I summarizes the findings as regards dental structure. The term M-hypoplasia refers to roughness of the buccal enamel compared with the enamel texture of puppies fed on diets relatively rich in vitamin D and containing the necessary calcium and phosphorus, such hypoplastic defects having been found to be closely related to caries susceptibility (Mellanby, 1918, 1923, 1929, 1930, 1934, 1936; Board of Education, 1931; King, 1940). It can be seen that, while few children even now have a full complement of perfectly calcified teeth, there has been a considerable change for the better during the past fourteen years. In Table II the findings are given when the schools are divided according to their estimated social status. There are no great differences in these groups in 1943, although the tendency is for the highest grade schools (B +) to have teeth of rather better structure than the schools in the other three grades. In 1929 there was a suggestion that the quality of structure was worse in the lower-grade schools.

TABLE I—INCIDENCE OF M-HYPOPLASIA IN DECIDUOUS TEETH

	Total children	Percentages showing:			
		none	little	some	much
1943	1,571	1·2	18·1	47·4	33·3
1929	1,139	0	7·8	33·6	58·5

TABLE II—INCIDENCE OF M-HYPOPLASIA IN DECIDUOUS TEETH AT THE DIFFERENT SOCIAL LEVELS

Social grading of school	Total children	Percentages showing:			
		none	little	some	much
Highest grade (B +)					
1943	190	0·5	25·8	49·0	24·7
1929	133	0	12·8	30·8	56·4
2nd grade (B)					
1943	582	1·0	14·8	50·2	34·0
1929	423	0	9·9	36·4	53·7
3rd grade (B −)					
1943	556	1·8	18·0	45·1	35·1
1929	190	0	4·7	30·0	65·3
4th grade (C)					
1943	243	0·8	20·2	44·9	34·2
1929	393	0	5·4	33·3	61·3

In Table III the incidence of dental caries in the two investigations is set out, the examination criteria being as far

TABLE III—INCIDENCE OF DENTAL CARIES IN DECIDUOUS TEETH

	Total children	Percentages showing :			
		no caries	little caries	some caries	much * caries
1943	1,604	22·4	25·9	22·4	29·3
1929	1,293	4·7	11·7	20·8	62·8

* Only 6 % of the children in this category in 1943 would correspond with the C6 group (very bad caries), compared with 54 % in 1929.

as possible identical. It is clear that a considerable improvement in dental health has occurred since 1929. Actually the improvement is even greater than might appear at first sight, as in 1943 only about 6 % of those in the " much caries " group could be classed in the " very bad caries " group (differentiated in 1929) compared with 54 % in the earlier study, which included a number of edentulous children. Separation of the children according to social level (Table IV) indicates that in 1943 there was distinctly less caries in the B + schools than

TABLE IV—INCIDENCE OF DENTAL CARIES IN DECIDUOUS TEETH AT THE DIFFERENT SOCIAL LEVELS

Social grading of school	Total children	Percentages showing :			
		No caries	Little caries	Some caries	Much caries
Highest grade (B +)					
1943	193	29·5	28·5	21·2	20·7
1929	147	6·1	17·7	15·6	60·6
2nd grade (B)					
1943	596	20·8	27·7	22·0	29·5
1929	480	6·5	11·3	22·6	59·6
3rd grade (B −)					
1943	569	22·9	23·6	21·1	32·5
1929	210	3·8	10·5	22·9	62·8
4th grade (C)					
1943	246	19·5	25·2	27·2	28·1
1929	456	2·8	10·5	19·5	67·1

in the other three grades. In 1929 there was a slight tendency for caries to increase from the better to the poorer schools. In addition to the main survey, a subsidiary group of 266 children, not comparable with those in any of the 1929 schools, was examined in 1943 ; the findings were in general similar to those in the main 1943 investigation.

Other results, not considered in so much detail, concern *arrested caries* and *association of M-hypoplasia and caries*. It was found that, in at least some teeth, 28 % of the 1943 subjects showed evidence of arrest of the carious process. An indication was also obtained that some post-eruptive influence (direct or indirect) appeared to have qualified to some extent the previous close association between the surface structure of the teeth and their susceptibility to decay.

In a discussion of the results the authors summarize their findings as follows :

" The data obtained in this survey show clearly that there has been a distinct improvement since 1929 in the dental condition of 5-year-old children attending London County Council elementary schools. The quality of the teeth is now better ; in 1943, 19 % of the children had teeth of good structure, compared with 8 % in 1929, while the percentages with very defectively formed teeth (i.e., teeth with ' much ' M-hypoplasia) were 33 and 58 respectively. The reduction in the incidence of dental decay is even more striking : in 1943, 22 % of the children were ' caries-free,' compared with 5 % in 1929, while only 29 % had ' much ' caries in 1943 as compared with 63 % in 1929. When the schools were grouped according to social status there was little difference in dental condition as regards both structure and caries between the various grades, though the teeth tended to be rather better in the highest-grade schools in 1943 and in the two highest grade schools in 1929."

They go on to point out that, as the teeth of the 1943 5-year-old children were in the main formed in the immediate pre-war years and the first year of the war, the cause of the improvement in these particular children must be sought among factors operative at and since that time, and contrasted with the conditions in the years 1922–29, when the teeth of the children examined in the previous survey were developing. The possibility that the changed dietary, including increased intake of vitamin D and calcium, the provision of cheap milk and cod-liver oil to infants and young children, together with the addition of vitamins A and D to the margarine and of calcium carbonate to the bread, has played a significant part in decreasing caries—susceptibility is also discussed. In this respect the improved dental structure would seem to afford some evidence, but the authors are inclined to believe that some other unknown factors have also been at work. Among the latter they mention the possibility that changes in the carbohydrate intake or a greater use of the tooth-brush may be here involved but rightly point out that " the basis of these suggested factors [*i.e.* carbohydrate intake and tooth-brushing] is too insecure at the present time to allow their effects to be assessed adequately." In conclusion, the authors state that " It is possible that the elimination of dental caries may not be attainable until its immediate cause is known, but even without this knowledge it is probable that a continuation and extension of the present nutritional policy and its more wholehearted adoption by the public would bring about further improvement in the architecture of the teeth and in their subsequent resistance to decay."

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FLUOROSIS AND DENTAL CARIES ON TYNESIDE

by R. Weaver, *British Dental Journal*, **76**, 29-40, 21/1/44

The teeth of 500 children aged five years and 500 children aged twelve years were examined in each of two towns in North-East England. Special attention was paid to the incidence and extent of dental caries and enamel mottling. Two methods were used for recording *caries* : (i) a modified form of that employed by M. Mellanby (1934), which allowed the comparison of groups of subjects by means of " average caries figures " ; and (ii) another adapted from American workers in which the conditions found were expressed as the average number of teeth per child which were decayed, missing or filled (" average DMF "). For *mottling*, the criteria were as follows : (*a*) no mottling ; (*b*) questionable mottling, paper-white patches on the enamel of only one tooth ; (*c*) very slight mottling, similar patches involving less than 25 % of the surface of two or more teeth ; (*d*) slight mottling, similar conditions involving 25–50 % of the enamel surface of two or more teeth. The more severe forms of mottling, including pitting, brown staining and change in tooth contours, were not found.

The *state of nutrition* of the children was also roughly assessed according to the four nutritional categories suggested some years ago by the Board of Education, viz. : A, excellent ; B, normal ; C, slightly sub-normal ; and D, bad. Finally, the water supplies for the two towns were analysed with particular reference to their fluorine content, and an account was given of the economic, sociological, geographical and other conditions obtaining in North and South Shields.

Results : The towns from which the examination subjects were selected were only about 500 yards apart, being separated by the River Tyne, but a considerable difference was found in the caries susceptibility. The only other difference which the author could determine was in the fluorine content of the water supplies of the communities north and south of the river. In North Shields, the drinking water contained less than 0·25 parts per million of fluorine ; in South Shields the water contained approximately 1·4 parts per million of

fluorine. The detailed results may be briefly summarized as follows:

i. Extent of caries in deciduous teeth of 5-year-old children.

	North Shields	South Shields
Number of teeth considered ...	3,279	1,951
Average caries figure	16·0	9·5
Average DMF	6·6	3·9

ii. Extent of caries in permanent teeth of 12-year-old children.

	North Shields	South Shields
Number of teeth considered ...	2,133	1,195
Average caries figure	10·0	5·1
Average DMF	4·3	2·4

The incidence of dental caries in the permanent teeth in South Shields was only 56 % of that in North Shields.

iii. No case of definite mottling of the *deciduous* teeth was seen in any of the 1,000 children examined.

iv. No relationship could be found between dental caries and mottling, as shown for the *permanent* teeth of 500 children:

	Number of children	Number caries-free	Average caries figure	Average DMF
Mottling absent ...	366	104	5·0	2·3
„ questionable ...	28	3	6·1	2·7
„ very slight ...	88	18	5·5	2·6
„ slight ...	18	4	5·3	2·3

The author then discusses his findings with reference to those of previous workers and, though he appears to believe that the higher fluorine concentration in South Shields was related to the lower caries susceptibility, he makes no suggestion as to how the increased resistance of the teeth is brought about.

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FLUORINE AND DENTAL CARIES: FURTHER INVESTIGATIONS ON TYNESIDE AND IN SUNDERLAND

by R. Weaver, *British Dental Journal*, **77**, 185–193, 6/10/44

In a previous paper (Weaver, 1944) the author reported the low incidence of dental caries in children of South Shields as compared with the incidence in the neighbouring town of North Shields. The drinking water in South Shields had a higher fluorine content. Examination of available statistics showed no evidence of superior general health of the South Shields population. A further survey of the dental condition of children of 14 years and over showed smaller differences in the incidence of caries in areas having a water supply of differing fluorine content. The incidence of untreated caries in mothers from North Shields and South Shields did not differ appreciably.

The author concludes that fluorine (if this is indeed the caries-inhibiting factor) postpones, but does not prevent, dental caries. He argues from data presented in the paper that the caries-inhibiting factor exerts its influence on the teeth during the pre-eruptive period. If this is true, the rational basis for the topical application of fluorine solutions to the teeth in caries prophylaxis would seem to be doubtful.

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[1] Weaver, R. (1944) *Brit. dent. J.* **76**, 29

[1] [*see BMB* 527]

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BLEEDING GUMS AND GINGIVITIS IN NAVAL RATINGS

by A. C. Macdonald, *Lancet*, **2**, 697–699, 4/12/43

In this paper are reported the results of a survey of the incidence of bleeding gums and gingivitis in otherwise physically fit naval personnel on active service in three small sea-going ships and a depôt ship. The men of the largest of the three sea-going craft were observed over a period of 14 months, the other three groups on only one occasion. All the subjects were on a full service diet and had a considerably better fruit and vegetable content of the diet than the members of the armed forces or civilians resident in England. Whilst at their bases abroad daily issues of fruit juices were made, and in the two smaller ships the men were encouraged to take multivitamin tablets [composition not stated]. In all, 614 men were examined, of whom 6·5 % were edentulous, 21·8 % showed a mild gingivitis and 6·7 % a severe gingivitis. The incidence of gingivitis was higher in the sea-going ships and in the over-30-years age-group. In the group of 156 men kept under observation for 14 months, 17 men reported their condition spontaneously; of these 11 had gingivitis, 4 were cases of acute ulcerative Vincent's infection of the gums and 2 had both symptom-complexes; all of these last 6 cases had a history of sore and bleeding gums. Examination of the whole ship's company disclosed 24 more cases of gingivitis, of whom 6 were undergoing treatment for syphilis with intravenous organic arsenicals.

Treatment consisted in removal of the tartar by scaling. Intravenous arsenicals gave a good response in 8 days in acute ulcerative gingivitis or in those cases with faucial infection. The chronic cases responded most readily to 10 % chromic acid followed in one minute with hydrogen peroxide (10 vol.) applied on cotton-wool pledgets. A normal mucoperiosteal junction was obtained in from 5–7 days in mild cases, and 7–12 days in the severe forms.

During a two months' observation a 22 % recurrence was observed.

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GINGIVAL DISEASE IN GIBRALTAR EVACUEE CHILDREN

by J. D. King, A. B. Francklyn & I. Allen, *Lancet*, **1**, 495–498, 15/4/44

In the autumn of 1941 a high incidence of fairly severe gingivitis was found by Francklyn (1941, 1942) in a group of 946 children evacuated to London from Gibraltar. As a result of this finding, Dr. King undertook, at the request of the Ministry of Health, a more critical investigation in a smaller group of 135 Gibraltar children of 10–14 years. Of these, 40 % were affected by lesions simulating the earlier phases of ulcerative gingivo-stomatitis (Vincent's disease) as compared with 1–2 % of 403 English children. The fact that the lesions were prevalent in groups of Gibraltarians living in widely separated parts of London, between whom there was little or no contact, precluded an infective basis for the disease. Moreover, although these children attended London elementary schools and mixed with the English pupils, the latter were singularly free from the ulcerative disease. General mouth hygiene, occlusion of the teeth, incidence and extent of caries, and tartar deposition did not differ significantly from such conditions in more than 2,500 English children examined by King at various times during the past six years. No obvious evidences of deficiency of vitamins A, C or riboflavin could be found. Since, therefore, the resemblance to Vincent's disease seemed to be the most likely clue to the nature of the disorder, it was decided to carry out a therapeutic trial of the effects of nicotinic acid administration, previously found to be of value in the treatment of gingival ulceration in adults (King, 1940a, 1943). Lack of co-operation on the part of both parents and children led to restriction of the number of subjects for the test. Eventually, 36 boys receiving the same basal dietary were divided into four groups as follows:

Group A. Each of 10 affected children was given 50 mg. of nicotinic acid daily for the five school days of two successive weeks: the vitamin was swallowed in tablet form at approximately the same time each day during the school morning session.

Group B. Each of 11 affected subjects received local hygienic treatment for five consecutive days of one week, as follows:

1st day: Application of chromic acid (10 %) to all gingival tissues, healthy as well as diseased, followed immediately by vigorous rinsing of the mouth with 10-vol. H_2O_2 (1 : 4 dilution).

2nd day: Removal of tartar by scaling, followed by further application of chromic acid and rinsing with H_2O_2.

3rd day: Removal of any remaining tartar and vigorous syringing with H_2O_2.

4th & 5th days: Syringing with H_2O_2.

Group C. Each of 10 affected boys was given 50 mg. of nicotinic acid daily for the five school days of two successive weeks plus the local hygienic treatment (as for group B) for the five school days of the first week.

Group D. A further 5 boys affected by the disease formed an untreated control group. Precautions were taken to prevent their receiving any medical or dental treatment elsewhere.

The children were carefully examined and detailed records were made of their mouth condition (*a*) before the test period, (*b*) two weeks after the test began, and (*c*) four weeks after completion of treatment. As the investigation was on a relatively small scale, the results were expressed in terms of the individual gum regions rather than of the mouth as a whole. The method of classifying the lesions was developed from that used by King (1940b). The findings are briefly summarized in Table I, where gingival disease is shown as M + (slight marginal), M + + (more marked marginal), P + (slight blunting) and P + + (more marked papillary disease involving slight ulceration).

TABLE I—RESULTS EXPRESSED IN TERMS OF COMPLETE RECOVERY OF GUM REGIONS ORIGINALLY AFFECTED BY THE FOUR GRADES OF DISEASE

A = Nicotinic acid; B = Local hygienic; C = Combined treatment; D = Untreated controls

Groups	No. of regions originally affected	Regions showing complete recovery			
		After 2 weeks [1]		After 6 weeks [2]	
		Number	%	Number	%
M+ Lesions					
A	15	10	66·7±12·17	15	100·0
B	35	31	88·6± 5·37	32	91·4±4·74
C	21	19	90·5± 6·40	21	100·0
D	6	0	...	0	...
M+ + Lesions					
A	52	30	57·7± 6·85	36	69·2± 6·40
B	49	22	44·9± 7·11	33	67·3± 6·70
C	69	57	82·6± 4·56	64	92·8± 3·11
D	31	0	...	3	9·7± 5·32
P+ Lesions					
A	23	12	52·2±10·42	14	60·9±10·02
B	41	9	22·0± 6·47	10	24·4± 6·71
C	22	4	18·2± 8·23	12	54·5±10·62
D	14	0	...	0	...
P+ + Lesions					
A	28	6	21·4± 7·75	11	39·3± 9·23
B	13	0	...	0	...
C	19	6	31·6±10·67	6	31·6±10·67
D	10	0	...	0	...

[1] [At completion of treatment; untreated controls re-examined on same date.]

[2] [At follow-up examination (no further treatment).]

This table shows the percentages of gum regions originally affected which completely recovered after 2 and 6 weeks respectively; the figures are tabulated to indicate the relative effects of the three treatments compared with the controls for each type of lesion. Local hygienic measures produced good results in the slight (M +) lesions but were less effective as the severity of the disease increased. In the most severe (P + +) lesions, local treatment produced no complete recovery, whereas the nicotinic acid alone and the combined treatments showed a significant 21 % complete recovery. It was, however, necessary to obtain some finer assessment of improvement or otherwise than that indicated by complete recovery. It was observed that in untreated cases progression from M + to P + + disease often occurred, so that by marking "normality" as 0, M + as 1, M + + as 2, P + as 3, and P + + as 4, a total disease figure per mouth could be calculated; from this an average disease figure was obtained by dividing the total disease figure by the number of regions which were or could be affected. Table II gives the average disease figures obtained by this method for

TABLE II—COMPARISON OF AVERAGE DISEASE FIGURES

(M+ = 1, M + + = 2, P + = 3, and P + + = 4)
A = Nicotinic acid; B = Local hygienic; C = Combined treatment; D = Controls

Group	At 1st exam. (*a*)	After 2 weeks (*b*)	After 6 weeks (*c*)	Difference between (*a*) and (*b*)	Difference between (*a*) and (*c*)
A	1·21	0·40	0·41	−0·81±0·10	−0·80±0·11
B	1·20	0·47	0·44	−0·73±0·10	−0·76±0·10
C	1·19	0·26	0·22	−0·93±0·09	−0·97±0·09
D	1·15	1·45	1·33	+0·30±0·17	+0·18±0·17
	Comparing A and D ...			1·11±0·19	0·98±0·20
	Comparing B and D ...			1·03±0·19	0·94±0·19
	Comparing C and D ...			1·23±0·19	1·15±0·19
	Comparing A and B ...			0·08±0·14	0·04±0·15
	Comparing A and C ...			0·12±0·14	0·17±0·14
	Comparing B and C ...			0·20±0·14	0·21±0·14

(*a*) initial examination, (*b*) after two weeks, and (*c*) after six weeks; and the differences between (*a*) and (*b*) and between (*a*) and (*c*). The controls, so far from showing improvement, deteriorated and, comparing them with each of the types of treatment, the differences are all statistically significant. For early lesions, local measures were effective but were progressively less satisfactory as the lesions became more severe. Treatment with nicotinic acid alone or combined with local hygienic methods gave better results than the local treatment alone, particularly for the more severe lesions; the results with nicotinic acid were, however, probably relatively delayed. The vitamin combined with local measures appeared to be more effective than nicotinic acid alone.

Finally a two-months trial of the effects of treating the lesions by administration of food-yeast (*Torula utilis*) was conducted in a further group of 41 affected Gibraltar children living in hostels on the same basal dietary. The test involved the daily addition to the diet of 3·5 g. of food yeast for 24 children; the remaining 17 controls received no treatment or supplement. The results (Table III), expressed more simply than for the main investigation, show that the yeast

TABLE III—EFFECT OF YEAST SUPPLEMENTS ON THE GUM LESIONS

Group	No. of children with different grades of gingivitis	
	before test	after test
E. Yeast supplement, 3·5 g. daily (24 children) [1] ...	P ... 13	P ... 2 M+ ... 3 Nil ... 8
	M+ + ... 5	M+ ... 1 Nil ... 4
	M+ ... 6	M+ ... 1 Nil ... 5
F. Untreated controls (17 children) [1]	P ... 11	P ... 6 M+ + ... 4 M+ ... 1
	M+ + ... 1	M+ + ... 1
	M+ ... 5	M+ + ... 3 M+ ... 1 Nil ... 1

[1] [In these children the P + and P + + lesions were not separated. The condition here recorded for each child refers to the most severe gum disease present in one or more of the regions examined.]

supplements considerably improved gingival health, but it was impossible to determine whether the beneficial effects were due to the small nicotinic acid content (1·4 mg. daily) or to some other component.

The authors conclude that the findings of the investigation as a whole indicated that deficiency of the nicotinic acid component of the vitamin B_2 complex played some part in the ætiology of the ulcerative lesions in the Gibraltar children studied, but that other undetermined factors existed, more particularly in the milder M-type disease. In support of the latter belief, the main results of an unpublished investigation (by J. D. King) of *non-ulcerative* gingivitis amongst young factory workers are cited. In an untreated control group, 50 % of 32 M + and 3 % of 32 M + + lesions recovered during an observation period of four weeks. The respective recovery figures in two other groups receiving nicotinamide (200 mg. daily) and ascorbic acid (200 mg. daily) for two weeks were 91 % of 54 and 63 % of 55 for the M + lesions, and 67 % of 41 and 17 % of 24 for the M + + lesions.

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[1] King, J. D. (1943) *Brit. dent. J.* **74**, 113, 141 & 169

[1] [*see BMB* **104**]

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INCIDENCE OF GINGIVITIS IN THE ROYAL AIR FORCE

by G. A. Smart, *British Medical Journal*, **2**, 242–244, 19/8/44

The author carried out a survey of the gums of random samples of airmen and airwomen from each of four Royal Air Force stations. The incidence of " gingivitis " was approximately 40 % of the 1,204 subjects examined, according to the criteria adopted. Of the affected personnel, 75·4 % were said to have non-ulcerative gingivitis, 8·9 % healed gingivitis, 15·0 % chronic ulcerative gingivitis, and 3·0 % acute ulcerative lesions superimposed on pre-existing inflammation. Unfortunately, while a detailed description is given of the meanings of these disease terms, the standard used for " normality " is less clearly indicated. Moreover, the author considers that one of the characteristics of healed gum lesions is absence of the interdental papillæ. This would imply that the disease has to progress to atrophy of these structures before signs of healing become evident, which is clearly not true in many cases. Apart from one bomber station where the women's gums were worse than the men's, no significant sex differences were found. As would be expected, the incidence of gingival disease became higher as age increased. An indication was also found that the pre-Service social status of the personnel had some influence on susceptibility to gum disease, the condition being less prevalent in the higher-income groups, and the author suggests that both dietetic factors and differences in personal hygiene may be concerned here. Finally, with regard to differences in gingivitis incidence among different categories of personnel, gum disease in the air-crews was significantly less than in the other groups. On the other hand, the incidence among those engaged in the preparation of food and associated duties was found to be significantly higher than among the remainder, excluding air-crews. He then remarks: " If the condition is infectious this finding is obviously important," but up to the present time, at least, there is no reliable evidence that most gingival lesions are infective and indeed there is much evidence to the contrary, so that this finding would scarcely seem relevant. There seemed to be no relation between the incidence of gingivitis and length of military service, but significant differences were found among the various trade groups, the incidence being lower among those who were more highly paid. No comments are made concerning the possibility that the relative amount of dental treatment received by the examinees might have had some influence on the gingival condition in the various groups. Some comparisons are made between the general findings and the investigations of gingival disease made by Semple, Price-Jones & Digby (1919), Whittingham (1932), Roff & Glazebrook (1940) and Macdonald (1943) among different Service units, in which the incidence figures ranged from 17·6 % to 28·6 % (uncorrected for age variations) according to the varying examination methods employed. In the author's results a method of age-correction was used.

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[1] [*see BMB* **529**]

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INCIDENCE OF BLEEDING GUMS AMONG R.A.F. PERSONNEL AND THE VALUE OF ASCORBIC ACID IN TREATMENT

by W. P. Stamm, T. F. Macrae & S. Yudkin, *British Medical Journal*, **2**, 239–241, 19/8/44

An investigation was made between October 1941 and May 1942 for the purpose of ascertaining the incidence of bleeding gums in the Royal Air Force and of assessing the value of ascorbic acid in the treatment of this condition. In addition, the ascorbic acid content of the food as served to the airmen in the messes was determined by the dichlorophenolindophenol method of Harris & Olliver (1942) during one week of the experimental period at each of the three stations considered.

Experiment i: The total number of personnel examined at the three stations was 1,791, and of these 344 (19·2 %) had some degree of bleeding of the gums. The incidence varied between the stations and between different classes at the same station, but not to any significant extent. Of those with bleeding gums, 119 were given 200 mg. ascorbic acid daily for 7 days followed by 100 mg. daily for 14 days; and 131 received dummy tablets flavoured with tartaric acid, forming the control group. Precautions were taken to ensure that no other form of treatment was given to these men during the experimental period.

No significant difference could be found between the two groups; ascorbic acid did not have a more beneficial effect than the dummy tablets, and indeed the latter had a slightly better effect. It was also of interest that in 5 of the 119 subjects having ascorbic acid, and in 9 of the 131 controls, ulcerative gingivitis developed during the experimental period. The average daily intake of the airmen at this time (Oct.–Nov. 1941) was 26 mg. ascorbic acid; later, in the Spring of 1942, the intake was 17 mg.

Experiment ii: At another Royal Air Force station, in April 1942, 122 out of 571 airmen (21·3 %) had bleeding gums. Of the affected number, 68 were given 150 mg. ascorbic acid for 20 days, and 48 who received the dummy tablets served as controls. Again, the dummy tablets were rather more effective than the vitamin supplements.

Experiment iii: At the same station as in experiment ii, 600 other airmen were examined for bleeding gums three times at intervals of three weeks during April and May 1942. Almost exactly the same percentage showed bleeding at each examination (about 20 %), but only 57 of the 122 with hæmorrhages at the initial inspection had bleeding at the final one. At the second examination the gums of 48 subjects (39 %) had stopped bleeding entirely—about the same number as showed improvement in experiments i and ii—but at the same time 37 of the remainder had started to bleed. Similarly, at the third examination 34 had stopped bleeding while 41 had started. The authors felt justified in concluding that in Royal Air Force personnel in this country ascorbic acid is valueless in the treatment of bleeding gums, and that the incidence of such lesions is not related to the ascorbic acid intake.

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STOMATITIS DUE TO RIBOFLAVIN DEFICIENCY

by H. E. Jones, T. G. Armstrong, H. F. Green & V. Chadwick, *Lancet*, 1944, **1**, 720–723, 3/6/44

In a North African camp containing 10,313 men of various races (Arabs, Berbers, Negroes, Ethiopians, Turks and

Europeans), 1,746 (16·93 %) were found to be affected by a form of stomatitis. The signs and symptoms included sore tongue, sore lips, some degree of trismus from the cheilosis, and excessive salivation; except for their mouths, the patients felt in excellent health. The conditions cleared rapidly on addition of milk, meat and eggs to the diet, or on administration of riboflavin or yeast.

Clinical findings: Lesions of the tongue began with sore, reddened areas at the tip and/or edges of the organ, where the papillæ were less conspicuous than usual. This process then spread backwards in a triangular pattern with the base of the triangle at the tip and the advancing apex central and pointing towards the root of the tongue; small areas of atrophy were often present in the unaffected areas. As atrophy advanced, the redness diminished, and during this intermediate stage alterations of the papillæ were in general of two types: (*a*) enlarged, pale, flat-topped filiform papillæ ("white mushrooms"); and (*b*) enlarged, less flattened fungiform papillæ with red centres ("red mushrooms"). In more advanced cases the tongue became increasingly smooth and shiny and fissures began to develop, radiating outwards from the median raphe. Sometimes, however, fissures were seen at much earlier stages or small, shallow, painful ulcers occurred. In the final stage the anterior part of the tongue was completely smooth and atrophic, with loss of papillæ, wasting, sharpened edges and tooth indentations. Occasionally a few petechial hæmorrhages were observed on the under surface of the organ, but the magenta colour of the tongue described by others as characteristic of riboflavin deficiency was not seen. In their later phases the tongues did not apparently differ from those of pellagrins.

In addition to the usual clinical examination, the slit-lamp microscope was employed for studying the above changes in the tongue papillæ as compared with "normal controls." With the aid of this instrument the authors found that in the affected subjects the characteristic condition was denudation of the filiform papillæ of their filamentous processes and subsequent atrophy of the papillæ themselves. The slit-lamp microscope showed the earliest alteration (loss of the filiform processes) in tongues which might appear macroscopically normal and might indeed have unusually prominent papillæ, giving a false impression. The tongues were divisible into two groups, atrophic and hypertrophic, of which the atrophic were much more common. The tip of the tongue showed the same changes in both groups, but there were differences in the central area of the organ. At *the tip*, both sets of papillæ were somewhat swollen and their margins came together, forming a more or less smooth pavement without denudation. The fungiform papillæ sometimes became opaque, with their contained capillaries hardly visible through the thinned and opaque epithelium covering them. The filiform papillæ lost their hairs and were seen as naked structures with opaque, pearly-white epithelium obscuring the blood-vessels; they began to resemble the fungiform papillæ in shape, making it difficult to differentiate between the two. In the *central area* the atrophic type showed three stages: (i) denudation of hairs; (ii) atrophy of the papillæ themselves; and (iii) complete disappearance of the papillæ, leaving an entirely smooth surface through which the linear streaks of the underlying vessels were indistinctly seen. For the hypertrophic variety of lesion the same changes occurred except that during stage (i) the filiform papillæ, besides losing their hairs, actually grew larger; they came to resemble the fungiform papillæ closely, but were distinguishable by their pearly-white opacity and the invisibility of their blood-vessels. Finally, sections from two affected tongues were compared with histological preparations from a normal one. The changes observed included flattening of surface papillæ, loss of ramification of down-growing epithelial processes, and complete cessation of epithelial growth.

As regards the lips and cheeks, the second important change in the syndrome described was an angular stomatitis which was not infrequently unilateral in distribution. This started as a tiny, painful, raw, red area at the commissure of the lips where the mucous membrane joins the skin. The red area spread along the mucosa of the cheek and lower lip but tended to be confined to a size of less than 1 cm.^{2}. Quite early this became covered with white epithelium (like blotting-paper), which could not be scraped off easily, and the latter sometimes extended to form large sheets, criss-crossed by cracks and resembling dried mud which had flaked. Concurrently, small painful fissures often developed in the skin at the corners of the mouth. The changes tended to spread to the mucosa of the lower lip, and in several cases fairly large, painful, oval ulcers developed. A less common finding on the lower lip comprised a group of small papular swellings, each of pin-head size and slightly paler than the surrounding reddened mucosa. These lip changes nearly always appeared near the skin margin, the gingival parts being spared. In only 20 of the 1,746 cases affected were lesions seen on the mucous membrane of the upper lip, and in all but one of these exceptions they were small. Some of the subjects with lips involved could be detected, before the mouths were opened, by virtue of a fine white line, due to epithelial alterations, visible from the angles of the mouth along the labial margins.

The incidence of lesions of the palate was about 5 %, a common site being well back on the hard palate, but occasionally there was a large area of redness near the alveolar margin. Their form was similar to those on the lips but their outline was always serpiginous and plaques were never seen; the edges were overhung by a rim of sodden, white epithelium.

Hospital investigation: A group of cases was then admitted to a British general hospital for more detailed study; their ages varied from 21 to 48 years. Routine and special examinations revealed the following findings:

None showed wasting, their skin was glossy and healthy and no pellagrous changes were seen; four had slight seborrhœic overgrowth in the nasolabial folds, but this sulphur-granule appearance also occurred in mild degree in 20 % of 300 controls; nails were well formed and strong, without koilonychia or fissuring; erythrocyte and leucocyte counts (20 cases) gave no data of significance; stools contained no excess of fatty acids or undigested food; sigmoidoscopy (10 cases) showed no abnormalities in bowel mucosa, and anal canals and perianal regions were normal; fractional test-meals (21 cases) indicated that free HCl was normal in 12 and high in 6, while 3 had achlorhydria; blood-urea (10 cases) varied from 18–42 mg. per 100 cm.^{3}; ten men, chosen at random, gave negative Kahn tests; eighteen out of 20 men had healthy gums; there was some sponginess in 2 and dental caries in only 1; seventy-five cases were examined for vascularising keratitis and all showed increased vascularity in mild degree, but all were suffering from trachoma (common in N. Africa); swabs from mouths of 30 cases showed moderate numbers of Vincent's organisms in 8 and monilia in 1; there was nothing to suggest that infection was a cause of the condition and local treatment with copper sulphate (5 %) "protargol" (5 %), and flavine (2 %) produced no improvement.

Relation to diet: Attention was then directed to the diet of the affected subjects, and it was noticed that the lesions began to appear about two months after dietary alterations had reduced the intake of certain food essentials; a table is given showing the approximate daily intake of constituents before and after the changes. It was found that the condition responded rapidly on adding milk, meat or eggs to the diet. Other remedies tried were:

Calcium lactate, given in 30-grain [about 1·8 g.] doses daily to 10 men for 10 days—no improvement;

Red palm oil, containing 3,800 international units vitamin A per ounce [about 28 g.], given in 1/10 ounce doses daily to 200 men for ten days—no improvement;

Oleum vitamini (B.P.), a dose containing 2,250 international units vitamin A and 250 international units vitamin D daily for ten days—no improvement;

Yeast, fresh or dried, given in doses of ½ ounce daily—rapid cure, causing the soreness to diminish or vanish within 4 days and most of the mouth changes to disappear in 7–10 days.

Thus the ætiological factor of the stomatitis was evidently related to deficiency of some constituent of milk, meat, eggs and yeast. Further trials showed that, while nicotinic acid was of no benefit in daily doses of 600 mg. for five days (5 cases treated), administration of riboflavin (10 mg. twice daily) effected striking improvement in 6 patients compared with 14 untreated controls. Finally, when the camp population was given a diet calculated to increase the riboflavin content to about 1·73 mg. riboflavin daily, the stomatitis ceased. [*N.B.* As the work was done in a desert hospital, no works of reference were available and the authors were unable to discuss their findings in relation to those of previous investigators in this field.]

Nutrition and Physical Fitness

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VITAMINS AND PHYSIOLOGICAL FUNCTION

by G. N. Jenkins & J. Yudkin, *British Medical Journal*, **2**, 265–266, 28/8/43

Harper, Mackay, Raper & Camm (1943) at Manchester demonstrated improvement in certain physiological functions in cadets who had been given a short period of supplementary feeding with vitamins. The present authors, of *St. Bartholomew's* Hospital Medical College and the *Dunn* Nutritional Laboratory, Cambridge, repeated these experiments on a group of Cambridge elementary-school children who had been receiving vitamin pellets for a year. Altogether 178 children were examined, comprising 80 12-year-old girls, 76 12-year-old boys and 22 11-year-old boys. About half in each group—alternate children from the school register—had received vitamin pellets daily at school, and the remainder received control pellets. The vitamin pellets contained 5,000 international units vitamin A, 1 mg. vitamin B_1, 25 mg. vitamin C and 500 international units vitamin D. The children were living and eating in their own homes except for some who had dinner at school. Allowing for week-ends and holidays, the average supplement over the year was roughly half the reputed daily requirements of these nutrients; apart from the vitamin B_1, which Harper *et al.* did not administer, the children were obtaining less of the supplemented vitamins than the subjects at Manchester. The functions tested were the same as those used by the Manchester workers, namely, average resting pulse-rate, average vital capacity, average vital capacity compared with body surface, average breath-holding time and average endurance test (the latter being represented by the time in seconds during which the subject could maintain, after full inspiration, a column of Hg at a level of 40 mm.). The children had also been measured for gain in height and weight, strength of grip, hæmoglobin, intelligence, educational attainment and dark-adaptation (Yudkin, 1943). There had been no improvement in these tests of the supplemented group over the control group.

The application to these children of the tests used by Harper *et al.* showed that there was no significant difference between the two groups. Half of the 12-year-old boys were also examined by the same tests three days after the completion of a vitamin-C saturation test, during which they received an average of 350 mg. daily of vitamin C. No difference could be detected between the saturated and the non-saturated group.

The authors emphasize that the absence of effect of the supplements in their subjects need not be interpreted as contrary to the findings of Harper *et al.*, but they indicate that the improvement of physical efficiency by vitamin supplements cannot be regarded as being generally applicable to all groups.

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[1] [*see BMB* **101**]

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LEVELS OF VITAMIN A AND C NUTRITION IN GLOSSOP SCHOOL-CHILDREN AND EFFECT OF DEFICIENCIES ON THEIR PHYSICAL CONDITION

by G. Kohn, E. H. M. Milligan & J. F. Wilkinson, *British Medical Journal*, **2**, 477–481, 16/10/43

This preliminary report from the Department of Clinical Investigation and Research, *Manchester Royal Infirmary* and the Public Health Department, Glossop, summarizes the results of an investigation covering 37 weeks between October 1941 and July 1942 on 400 school-children in Glossop between the ages of 9 and 14 years. All the children lived at home but had certain meals at school. They were divided into two groups. The "vitamin group" received 5 daily doses per week of 4,000 international units of vitamin A, 350 international units of "vitamin B complex," 1,000 international units of vitamin C, 600 international units of vitamin D, 2 mg. of riboflavin and 20 mg. of nicotinamide. The "control group" was given capsules containing no vitamins.

In a study of the vitamin-C levels of the children, the ascorbic-acid-saturation test formulated by Harris & Abbasy (1937) was used, and in some cases the ascorbic acid in whole blood was determined by the method of van Eekelen, Emmerie & Wolff (1937), which involves clearance with mercuric acetate and reduction of dehydroascorbic acid by H_2S. Three months after the beginning of the investigation, by which time the "vitamin group" had each taken an average of 3,000 mg. of ascorbic acid, all the 400 children were given two test doses of ascorbic acid (11 mg. per kg. body weight). All the "vitamin group," but only 28% of the "control group," were saturated. From April to June of the same year (1941) the vitamin-C intake in the diet was assessed as under 15 mg. per day in 40% and between 15 and 30 mg. per day in 35% of all children. The vitamin-C nutrition varied according to social standard but a relatively large amount of severe deficiency was found. Glossop children are said to compare very badly with school-children in other areas.

The incidence and degree of vitamin-A deficiency in the children was assessed by the methods described by Yudkin (1941); a threshold lability of 0·5 log unit or over was accepted as definite evidence of deficiency of vitamin A. On this basis, 16% of the children were deficient, 7% probably deficient and 15% possibly deficient. The daily dietary intake of vitamin A was assessed as over 50 international units per kg. body weight in nearly half the children; 20–30 international units per kg. in another 20%; and less than 20 international units per kg. in 7%. It was tentatively suggested that a dietary intake of over 30 international units per kg. can be described as the minimal daily requirement for children aged 9–14 years.

In the third part of the investigation as reported, five small groups of from 20–50 boys were selected for certain special tests, carried out over a period of 36·7 weeks, the results of which were analysed statistically. The diets of all these boys were carefully investigated and it was believed that the groups differed only in regard to their vitamin intakes; certainly there were no significant differences between the groups in age. Although there were no significant differences in percentage gains in height, a group of 20 vitamin-A deficient boys gained significantly less in weight than a group of 36 boys from the "vitamin group." In an endurance test involving the length of time that the subjects could hold themselves suspended from a horizontal bar, the performance of groups of boys deficient in vitamin A and in vitamins A and C significantly deteriorated during 36·7 weeks, and there was a significant difference between these groups and boys from the "vitamin group." No significant differences between groups were detected in strength (arm dynamometer) or in endurance, and saturation of C-deficient boys with ascorbic acid produced no improvement in their physical efficiency as determined in this way. It was concluded therefore that the short-term effects of ascorbic acid appear to be different from its long-term effects. Examination of groups of boys showed no significant difference in the incidence of gingivitis between C-deficient and "vitamin group," but the incidence of infection was significantly lower in a "vitamin group" than in both A-deficient and C-deficient boys. It was also considered at the end of the investigation that children of the "vitamin group" had "clearer, smoother and sleeker" skins than were found in other groups.

No significant differences between groups were found in respect of plasma phosphatase values (method of King, Haslewood & Delory, 1937) or serum complement titres (method of Ecker, Pillemer, Wertheimer & Gradis, 1938).

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THE EFFECT OF SUPPLEMENTS OF VITAMINS AND MINERALS ON THE HEALTH OF GIRLS

by H. Fowke, *British Medical Journal*, **2**, 519, 23/10/43

The investigation of which this paper forms a preliminary report was made between February 1941 and April 1942 under the direction of the Ministries of Food and Health. 214 girls between the ages of 8 and 12 years distributed between five institutions in the North of England were

divided into two approximately equal groups, one of which was given a daily ration of 1 ounce [about 28 g.] of " fortified chocolate " containing 2,000 international units of vitamin A, 250 international units of vitamin B_1, 100 international units of vitamin D, 35 mg. of vitamin C, 250 mg. of calcium and 10 mg. of iron. The control group of girls received similar chocolate to which nothing had been added. The dietary conditions in the different institutions varied and there was a general improvement in all diets, as assessed from tables, during the experimental period. Calculation of the calorie values and contents of animal protein, calcium, iron and vitamins A, B_1 and C, before the experiment began and again seven months later, showed that in two of the institutions the diets were deficient in all nutrients, marginal in two others, and probably adequate in the fifth. At the beginning of the investigation, after six months, and at the end of a year, a complete medical examination was made of each child. Data on the following points were obtained ; height, weight, sitting height, chest-circumference, the time the breath could be held, the lifting-power measured by a spring dynamometer, the time each child could hang from a horizontal bar. No statistically significant differences were found between the two groups in respect of any of these functions. This may have been due to a steady improvement in the basic diet during the investigation or to the fact that the tests used were insufficiently sensitive to detect minor degrees of malnutrition.

The investigation established that chocolate is a most satisfactory medium for the incorporation of vitamins and minerals. It is a food in itself, with a popular appeal and a pleasant taste strong enough to conceal that of the supplements. Tests of the keeping qualities of vitamins A, B_1 and C in specimens of chocolate stored at room-temperature for four months showed remarkable stability.

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THE INFLUENCE OF SUPPLEMENTS OF VITAMINS A, B_1, B_2, C, AND D ON GROWTH, HEALTH, AND PHYSICAL FITNESS

by E. R. Bransby, J. W. Hunter, H. E. Magee, E. H. M. Milligan & T. S. Rodgers, *British Medical Journal*, **1**, 77–78, 15/1/44

This paper contains a summary of the results of an investigation carried out in 1941–42 on approximately 1,400 schoolchildren and adults in order to determine whether growth, health and physical fitness could be improved by the addition to the diet of supplements of synthetic vitamins. The vitamin capsules employed were a gift to the Ministry of Health from a group of physicians in the United States and had the following composition: Vitamin A 4,000 international units, thiamin 333 international units, riboflavin 2 mg., ascorbic acid 1,000 international units, nicotinamide 20 mg., and vitamin D 600 international units. The vitamin content of the capsules was checked by the *Medical Research Council* four times during the tests.

Three of the five tests carried out were performed on 1,242 elementary school-children in Ipswich, Glossop and London, between November–December 1941 and July–August 1942. The children were examined before, during and after the administration of vitamin supplements and were divided at random into two equal groups, one of which received a capsule every school day and the other a control capsule of arachis oil. It was found that the administration of the capsules had no significant effect on rate of growth, nutritional status, muscular strength, condition of teeth and gums, or absence from school on account of illness.

The remaining tests were done on 214 adult male factory workers and were carried out over the same period and on the same general lines. The vitamin capsules were observed to have no significant effects on weight, hæmoglobin, blood-pressure, absence through illness, or output of work.

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GROWTH AT HOME AND AT SCHOOL

by E. M. Widdowson & R. A. McCance, *Lancet*, **2**, 152–153, 29/7/44

It has previously been reported by Allan (1937, 1939) that boys at " public schools " * in England gain weight more rapidly during the holidays than they do during the term. This paper from the Department of Medicine at Cambridge contains an analysis of the gains in weight and height of groups of boys in their thirteenth, fourteenth and fifteenth years in three well-known public schools. The boys were weighed and measured at the beginning and end of each term between 1938 and 1943, and the monthly increments of weight and height were calculated for each boy for three terms and three holiday periods. The mean results for all the boys were then compared.

In two schools out of three, the average monthly gains both in height and weight of 930 boys were invariably greater during the holidays than they were during the terms. During the 17 weeks of holidays these boys gained about twice as much weight as they did during the 35 weeks of term, but the increases in height were not so striking. At the third school (240 boys), the boys grew more regularly throughout the year and the most rapid gains tended to take place at school.

The authors point out that this irregular growth can hardly be attributed to a normal physiological mechanism. It may be that the greater mental and physical strain at school accounts for the diminished growth-rate: there was no reason to suppose, however, that conditions in the third school were any different from those in the other two, and the boys came from the same type of home. The authors feel that " it is difficult to get away from the view that the differences in growth-rate must ultimately be nutritional in origin." They conclude that " until we know more about the causes and consequences of these irregularities of growth, results such as these emphasize how necessary it is for boys to have good holidays, at home, free so far as possible from work, camps and all other kinds of forced labour."

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THE NUTRITIONAL STATUS OF CAMBRIDGE SCHOOL-CHILDREN

by J. Yudkin, *British Medical Journal*, **2**, 201–205, 12/8/44

This paper by Dr. John Yudkin, who formerly worked at the *Dunn* Nutritional Laboratory, Cambridge, contains the results of a survey of over 1,100 Cambridge children between the ages of 4 and 11 attending elementary schools. This represents nearly 20% of this age-group in the area. The nutritional status of the children was assessed in January and February 1942 and again in the same months in 1943. The results were found to compare favourably with those reported from other parts of the country. Compared with children in a better-class area, children from poor quarters of the area were on the average 0·8 in. [about 2 cm.] shorter, 2·6 pounds [about 1·3 kg.] lighter, had 2% less hæmoglobin, a grip about 1·25 kg. weaker and showed more changes in conjunctival epithelium when examined with the slit-lamp microscope (Kodicek & Yudkin, 1942). At the second examination, a year later, the heights and weights were slightly greater than for children of the same age examined the year before. It may be said, therefore, that there had been no deterioration in their diets between the third and fourth years of the war.

Further studies were later made on about 200 of the oldest children to determine whether " physiological performance " can be improved by the giving of vitamin supplements (for the results of this investigation see Jenkins & Yudkin, 1943). After the first examination, half the children at each school were given, each day, a pellet containing vitamin A 5,000 international units, vitamin B_1 1 mg., vitamin C 25 mg., and vitamin D 500 international units, and the other half received dummy pellets. Allowing for holidays and absences from school, the pellets supplied approximately half the daily requirements of vitamins A, B_1, C and D. After a year no effect was found in the gains in height and weight, hæmoglobin, strength of grip, dark-adaptation, resting pulse rate, vital capacity, breath-holding time or endurance as measured by the R.A.F. mercury test. There was, however, an improvement in vitamin-C saturation, a decrease in the incidence and duration of colds, and an improvement in school behaviour as assessed by the teachers. The author points out that " the absence of effect on growth, hæmoglobin,

* [a term used in Britain to denote the larger private schools, most of which are well endowed and of ancient foundation. Most pupils at these schools are residential.]

etc., cannot be taken to imply that these are not limited by nutritional factors."

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[1] [*see BMB* **534**] [2] [*see BMB* **33**]

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THE NUTRITIONAL STATUS OF CHILDREN AND MOTHERS OF INDUSTRIAL TOWNS

by J. Yudkin, *Medical Officer*, **72**, 93–94 & 101–102, 16/9/44 & 23/9/44

The author has previously reported (Yudkin, 1944) a nutritional investigation in Cambridge school-children. In the present paper he describes a more limited investigation on working-class mothers and children of 66 families from Aberdeen, Dundee, and Glasgow.

On the average, these children from industrial towns were nearly 1½ inches [about 3·5 cm.] shorter, weighed 4 pounds [about 1·8 kg.] less, had a hæmoglobin level 4% lower, and a strength of hand-grip 1½ kg. less than the Cambridge school-children. There was a correlation between the financial state of the family and the nutritional state (as judged by the tests described) of its members.

Although the best use was not always made of the income available for food, the author states that there was not in most cases sufficient money for an adequate diet. The data from this survey are summarized in 9 tables and 3 graphs.

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[1] [*see BMB* **539**]

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NUTRITION AND SCHOLASTIC ATTAINMENT

by I. F. MacKenzie, *British Medical Journal*, **2**, 205–207, 12/8/44

With the ultimate object of promoting education, the Education (Provision of Meals) Act was passed in 1906, granting local authorities powers to provide meals for children attending public elementary schools. Even to-day, however, defective nutrition is common, and the question arises whether malnutrition interferes with the educational progress of the child. Such evidence as at present exists suggests that there is a correlation between physical condition and mental development (Sandwick, 1920; Baldwin, 1922; Pearson, 1923; Habakkuk, 1926; Mann, 1926; Leighton & Clark, 1929; Dawson, 1931).

The author of this paper, who is Deputy County Medical Officer for Herefordshire, has examined the nutritional status of 712 children between the ages of 8 and 14 attending a group of public elementary schools. The assessment of nutrition was made on the findings of a clinical examination which followed that recommended by the League of Nations Health Organization (1938), and the classification used was that sponsored by the Board of Education (1934). The pupils were chosen by the teachers to represent the "bright" and the "slow" in comparison with the class averages. Of the bright children, 28·2% were graded Nutrition A and 5·6% Nutrition C. Only 6·7% of the slow children were grade A, while 22·1% were graded C. From a comparison of the results in 280 boys and 303 girls it was concluded that the mental activity of girls is even more closely related to their general physical state than is that of boys.

The author holds the view that "subnormal nutrition can constitute a serious handicap to the educational advancement of a child" and believes that a malnourished child may, as the result of proper feeding, "attain to an ability to use his mental powers equivalent to that enjoyed by his better-nourished school-mate."

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542

NUTRITION AND SIZE OF FAMILY

by J. Yudkin, *Lancet*, **2**, 384–387, 16/9/44

The author reports the results of comparative tests of physical condition in school-children from large and small families of (*a*) a poor quarter and (*b*) a more prosperous area of Cambridge. The height, weight, hæmoglobin levels, and muscular strength (hand-grip) of children from larger families in the poor area were significantly lower than those of children from small families. The differences between children of large and small families in the more prosperous area were smaller (taking into consideration the smaller disparity in size of family in this group).

The author concludes (i) that family income in the poor area is not sufficient to secure physical development for more than a limited number of children; (ii) that family allowances are necessary to improve the nutritional status of children of large families.

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ACCELERATION OF CO-ORDINATED MUSCULAR EFFORT BY NICOTINAMIDE: Preliminary Report to the Medical Research Council

by I. M. Frankau, *British Medical Journal*, **2**, 601–603, 13/11/43

This communication summarizes the results of a series of experiments undertaken to show by means of a selected test the effect, if any, of certain vitamins on the physical efficiency and the fatigability of healthy young males. Preliminary experiments demonstrated that the usual agility-test was not adequate to produce any measurable degree of fatigue in the subjects, who were drawn from the personnel of the *Royal Air Force*. The test finally adopted consisted of running rapidly for about 300 yards, bending and turning quickly and dropping discs accurately on a closely fitting shaft. No undue stress was laid upon speed or accuracy, but the need to finish with maximal exertion was emphasized.

Two control tests were recorded on one day at intervals of 30 minutes, and two "experimental" tests with an interval of 15 minutes on another day. No significant change was detected after ingesting on three successive days vitamin tablets containing vitamin A, 8,000 international units; vitamin D, 1,200 international units; aneurin, 2 mg.; riboflavin, 4 mg.; ascorbic acid, 100 mg.; and nicotinamide, 40 mg.

After 4 days' dosage with vitamin tablets which provided aneurin 5 mg., riboflavin 5 mg., ascorbic acid 100 mg. and nicotinamide 50 mg. per day, tests carried out on the fifth day showed a significant improvement in this group over the control group, both as regards co-ordination and endurance. Further experiments omitting aneurin and both aneurin and ascorbic acid showed that neither of these two substances was required to produce this effect.

Subsequently, experiments were carried out on the effect of nicotinamide alone. Control times were recorded on one day. On the following day each subject received 50 mg. of nicotinamide, and the test was repeated on one group after 1½ to 2½ hours and on a second group 5½ to 6½ hours later. Yet another group was given 200 mg. 1½ to 3 hours before the test. A significant improvement was observed in all subjects after nicotinamide, and the results were more evident at short intervals after the dose. Administration of nicotinamide for 6 successive days followed by tests on the seventh demonstrated the same general trend.

Diet and Disease: Communal Feeding

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NUTRITIONAL DEFICIENCY IN THE PATHOGENESIS OF DISEASE

by J. Yudkin, *British Medical Journal*, **1**, 5–7, 1/1/44

Dr. Yudkin, formerly *Sir Halley Stewart Research Fellow* at the *Dunn* Nutritional Laboratory, Cambridge, points out in this paper that certain criteria are necessary for assessing the

part played by nutritional deficiency in producing obscure signs and symptoms or syndromes of uncertain ætiology. These criteria are analogous to Koch's postulates and fall into three groups.

Criterion 1. *It should be possible to demonstrate the existence of a deficiency of the nutrient.* Evidence of such deficiency may be found at any point between the nutrient in the diet and its utilization in the tissues. A careful dietary history is therefore essential. Under this head are included such factors as income level, season, habitat, special dietary régimes, conditions of employment, standard of preparation and cooking of the food, etc. Evidence of defective absorption must next be considered and thirdly the possibility of increased utilization, such as occurs, for example, in pregnancy or as the result of fever or heavy manual work. Specific signs of deficiency disease may be obtained from clinical examination, and as the result of functional tests and/or laboratory investigations of blood and urine. Unfortunately, however, these methods have often been used uncritically; though superficially simple they require rigid control and careful interpretation.

Criterion 2. *Deficiency of the nutrient should result in the production of the disease.* This test may be applied in experimental animals, in which case the results must be evaluated with due care, or in human beings. Conflicting results are often obtained in human tests by different workers, possibly as the result of an inadequate number of subjects, individual variations in behaviour and requirement, and differences in the composition of the diet.

Criterion 3. *The disease should be cured by replacement of the deficient nutrient.* In assessing the value of a therapeutic test, however, it is important to allow for the possibilities (*a*) of spontaneous remission and conversely (*b*) that the pathological process may have progressed to an irremediable stage. The existence of multiple deficiencies is a less well recognized reason for failing to obtain improvement. Lastly, when a positive effect is finally obtained by the use of a vitamin supplement it is not always legitimate to assume that the existence of a deficiency has thereby been proved.

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EFFECTS ON RATS OF PROLONGED FEEDING WITH THE STAPLE AFRICAN DIET

by J. Gillman, *British Medical Journal*, **1**, 149–150, 29/1/44

It is known that pulmonary tuberculosis and other diseases of the respiratory tract are prevalent among South African negroes, in whom cirrhosis of the liver, and especially primary cancer of the liver, are encountered with unusual frequency. Malnutrition is widespread among the Africans, whose staple diet consists largely of maize meal and sour milk. This paper from the Department of Anatomy of the University of Witwatersrand, Johannesburg, represents the collaborative efforts of many workers in the Department who have investigated the effect of such a diet on normal rats. Full post-mortem examinations were made of 12 rats who had received liberal amounts of the diet for 14 months.

In all the animals, pronounced disease of the liver was evident macroscopically. This varied from diffuse enlargement of the whole liver with obvious fatty change to a widespread nodular cirrhosis often involving especially the left lobe. The hearts were grossly enlarged, fat deposition in the mesentery, retroperitoneal and subcutaneous areas was unusually abundant, and in 9 animals one or both lungs showed inflammatory lesions with bronchiectasis or abscess formation. Pathological changes were also found in the skull, teeth, pituitary, thyroid, suprarenals and alimentary tract. These striking abnormalities were present without any recognizable manifestation of acute vitamin-deficiency disease. No lesions of the liver of the kind described were ever observed in many thousands of rats examined post mortem over a number of years.

The author rightly points out that " the production of liver damage by means of a diet which forms the staple of the overwhelming number of Africans in South Africa is not without its sociological implications." Further work is in progress to determine the factors responsible for these remarkable changes.

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SOME PROBLEMS OF COMMUNAL FEEDING

by C. P. Stewart, *Edinburgh Medical Journal*, **51**, 215–228, May 1944

This is the text of a *Honyman Gillespie* lecture given in the Royal Infirmary, Edinburgh. The author points out " that the part played by communal feeding in the nutrition of our people is a very considerable one, and that a large fraction of the population depends upon it for the whole or part of its food." Community feeding provides an excellent field for research but the problems differ somewhat between the " closed " community (hospital, boarding-school, hostel, etc.) and the canteen and workers' restaurant. In both, however, there is the same need for a nutritional standard high enough to be of educational value, for variety, palatability and attractiveness in the food; for cooking and serving methods which will preserve the labile vitamins, and for economy without sacrifice of true value.

The author describes the results of a survey made in a boarding-school for boys aged 9 to 12 years, comparing his results with standards taken from the Report of the Hot Springs Conference and showing how closely practice may approximate to precept even with the food restrictions of war-time. A good diet, however, must satisfy other criteria than those of chemical analysis, and the boys' diet is further considered as to its variety, palatability and attractiveness. Cooking and serving cannot be assessed quantitatively, but objective evidence can be obtained on these points by determinations of the ascorbic acid content of the food as eaten. Figures are quoted for potato and cabbage for the school and *British Restaurants* (public restaurants instituted by the Ministry of Food) which reveal the better cooking and serving in the former and the great variation in the latter.

Dr. Stewart then discusses hospital diets, and shows that in many respects these must often be regarded as unsatisfactory. He found that patients in hospital were obtaining from 300 to as much as 1200 calories per day from food brought in by their friends.

Finally, the standards to be adopted for the " midday " (or " midnight ") meal in the factory canteen are discussed and it is shown how these may require to be adjusted according to habit, type of employment and local conditions.

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THE MIDDAY SCHOOL MEAL: A STUDY OF ITS RELATION TO THE TOTAL WEEKLY DIETARY OF A GROUP OF SCHOOLBOYS

by R. P. Cook, W. A. Davidson, D. M. Keay & D. G. McIntosh, *British Medical Journal*, **2**, 443, 30/9/44

This is a brief report of an investigation intended to show what proportion of the total intake of important nutrients was provided by the midday school meal of a group of 39 boys of 14–15 years who agreed to co-operate. Each boy recorded on a tabular sheet, which was provided, his food-intake at each meal for one week. Foodstuffs were divided into 20 categories, and their content of the nutrients considered was calculated from tables.

The mean percentage of the intake of calories obtained from the school meal was 35·68. The corresponding figures for important nutrients were : total protein, 35·92 ; " first-class protein," 44·4 ; vitamin A, 67·8 ; vitamin B_1, 37·13 ; ascorbic acid, 57·8 ; calcium, 42·3; available iron, 37·17. The authors regard the school meal as providing a valuable contribution to the dietary which would probably have been inferior if all meals had been taken at home.

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SULPHONAMIDES IN THE TREATMENT OF MENINGOCOCCAL MENINGITIS

Report to the Scientific Advisory Committee, Department of Health for Scotland. Edinburgh, H.M. Stationery Office, 1944. 20 pages. 4d. [£0.016]

During the early part of 1940, an exceptionally large epidemic of meningococcal meningitis, while giving rise to some anxiety, also gave an opportunity of a detailed assessment of the value of drugs of the sulphonamide group in the treatment of this infection. This report evaluates the relative efficiency of the various sulphonamides used. Certain hospitals were selected for the enquiry, and the report includes an analysis of all cases accepted by these hospitals between 1936 and 1941. As sulphonamides were little used before 1938, a comparison is possible with the results of treatment in pre-sulphonamide days. The records of 2,223 cases of meningococcal meningitis are analysed and it is shown that the use of sulphonamide drugs has caused a marked reduction in the fatality-rate (16·7 % as compared with 52 % in those receiving serum alone). Mortality was found to be closely associated with age, maximal rates occurring under 2 years and over 35 years. The method of treatment was as follows: no serum or drug, 12 cases; serum or antitoxin, 199; sulphanilamide, 128; sulphapyridine, 1,468; sulphathiazole, 75; miscellaneous sulphonamides, 91; serum or antitoxin and sulphonamides, 250. Comparison between sulphanilamide, sulphapyridine and sulphathiazole does not suggest that any one possesses clear advantages over the others. Between 70 % and 80 % of cases receiving sulphonamides were clinically cured within 4 weeks. The dosage given varied widely in individual cases and the analysis does not permit a dogmatic statement with regard to optimum dosage. The sulphonamides appear to be equally effective when given during any part of the first week of illness, although the prompt institution of treatment is important. Various complications were encountered; the importance of pneumonia as a cause of death is emphasized.

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MEDICAL DISEASES OF WAR

by Sir Arthur Hurst. 4th edition. London, Edward Arnold & Co., 1944. 511 pages; 48 illustrations. £1 1s. [£1.05]

This book originated during the 1914–18 war as a review of experience gained on active service on both western and eastern fronts. At the beginning of the present war it was one of the best available authorities on those medical diseases which become prominent, both among soldiers and civilians, in time of war. In 1940 appeared the first edition of the book in its present form, a modern, extensively revised version of the original work. This new book, which has reached its fourth edition in four years, reflects the wide reading and vast experience of the author. In its construction he has had the co-operation of several authorities. Particularly important is the section on the functional nervous diseases. In the present edition, the chapter on epidemic jaundice and infective hepatitis and the sections on sciatica and the treatment of bacillary dysentery have been rewritten. The section on the seborrhœic state has been replaced by one on dermatophytosis. Major alterations have been made in the sections on digestive disorders, malaria, tetanus, and the hysterical sequelæ of concussion, and minor corrections and additions are evident throughout the remainder of the work. The recent death of the author of this work is a great loss to British medicine.

Chapter headings: (i) predisposing causes of war neuroses; (ii) hysterical symptoms in soldiers; (iii) hysterical paralysis; (iv) hysterical contractures; (v) rheumatism, sciatica, and hysterical postures and gaits; (vi) hysterical tremor; (vii) hysterical fits; (viii) disorders of speech; (ix) functional disorders of hearing; (x) functional disorders of vision; (xi) hysterical stupor and amnesia; (xii) cerebral and spinal concussion; (xiii) exhaustion resulting in neurasthenia; (xiv) hyperadrenalism and hyperthyroidism; (xv) anxiety neuroses of war (by T. A. Ross); (xvi) digestive disorders in soldiers; (xvii) effort syndrome; (xviii) trench fever; (xix) louse-borne typhus fever (by Melville D. Mackenzie); (xx) typhoid and paratyphoid fevers; (xxi) dysentery; (xxii) epidemic jaundice; infective hepatitis; (xxiii) malaria (by H. B. F. Dixon); (xxiv) meningococcal fever (by A. W. Stott); (xxv) diphtheria (by E. H. R. Harries); (xxvi) tetanus; (xxvii) war nephritis; (xxviii) skin disease in war (by H. W. Barber); (xxix) gas poisoning.

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LECTURES ON DISEASES OF CHILDREN

by Sir Robert Hutchison & Alan Moncrieff. 9th edition. London, Edward Arnold & Co., 1944. 478 pages; 108 illustrations. £1 1s. [£1.05]

It is 40 years since this book first appeared, having originally consisted of lectures delivered by Sir Robert Hutchison in a systematic course at the *London* Hospital, of which he is Consulting Physician. This ninth edition is the responsibility of Dr. Alan Moncrieff, Physician to the Children's Department of the *Middlesex* Hospital and to Out-patients, *Hospital for Sick Children*, London. The book, written in didactic style, is essentially practical and clinical in its approach to the subject; detailed reference to treatment is a prominent feature. In this new revision, the principal alterations are to be found in the sections dealing with infection in the newborn, the treatment of infantile diarrhœa, the cause and treatment of cœliac disease, the treatment of threadworms, and the types of congenital heart disease, and sections on the Rh factor and on encephalitis have been added. This book is a standard British work on pædiatrics.

Chapter headings: (i) the clinical examination of sick children; (ii) infant feeding—breast-feeding; (iii) infant feeding—artificial feeding; (iv) some diseases of the newly-born; (v) the premature infant; (vi) diet after the period of infancy; (vii) the digestive disorders of infancy—colic and vomiting; (viii) congenital pyloric stenosis; (ix) infantile diarrhœa; (x) cœliac disease; (xi) chronic constipation in infancy and childhood; (xii) wasting; (xiii) congenital syphilis; (xiv) tuberculosis in childhood; (xv) rickets; (xvi) infantile scurvy and pink disease; (xvii) the dyspepsias of childhood; (xviii) rheumatism in childhood; (xix) acute rheumatic carditis; (xx) some disorders of the heart in childhood; (xxi–xxiv) the respiratory diseases of children; (xxv) some behaviour problems in childhood; (xxvi) some functional nervous diseases of childhood; (xxvii) convulsions in childhood; (xxviii) the paralyses of childhood; (xxix) meningitis and encephalitis; (xxx) on mental deficiency in childhood; (xxxi) the blood disorders of early life; (xxxii) chronic splenomegaly in childhood; (xxxiii) some common affections of the genito-urinary system in childhood; (xxxiv) affections of the liver in childhood; (xxxv) some common symptoms of disease in children and their diagnostic significance; (xxxvi) the diagnostic significance of abdominal pain in childhood; (xxxvii) fever of obscure origin; (xxxviii) some of the commoner skin diseases of infancy and childhood; (xxxix) disorders of growth and development.

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AN INTRODUCTION TO PHYSICAL METHODS OF TREATMENT IN PSYCHIATRY

by W. Sargant & E. Slater. Edinburgh, E. & S. Livingstone, Ltd., 1944. 171 pages. 8s. 6d. [£0.425]

Increasing knowledge of the ætiology of mental disease has resulted in a shifting of emphasis from psychogenetic to physical interpretations. Symptoms once thought to be of exclusively psychogenic origin have now been found to rest on an underlying physical deviation from the normal. The past decade has seen important advances in somatic methods of treatment in psychiatry (although they are still admittedly empirical), and this book, whose joint authors are medical officers to the *Maudsley* Hospital, London, provides a valuable and timely introduction to the subject. It is not a complete text-book, but will give a balanced view of the scope of physical methods of treatment. The writers, who have had special opportunities of applying these methods in the treatment of civilian and military psychiatric casualties and therefore write with authority, consider that it contains sufficient material to provide a basis for active treatment in the psychiatric ward of a general hospital or in the reception and treatment wards of a mental hospital.

The chapter headings are: (i) the insulin treatment of schizophrenia; (ii) modified insulin therapy; (iii) convulsion therapy; (iv) the treatment of cerebral dysrhythmia (bromides, luminal, epanutin, benzedrine); (v) chemical sedation and stimulation; (vi) continuous sleep treatment; (vii) some special uses of intravenous barbiturates; (viii) diet, vitamins and endocrines; (ix) prefrontal leucotomy; (x) the malarial treatment of general paralysis; (xi) the relation of psychological to somatic treatment.

An important literature has grown up around this subject, and the authors would still further increase the value of this book by the addition, in subsequent editions, of a bibliography of the more pertinent references.

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HOUSING MANUAL 1944

Prepared by the Ministry of Health and the Ministry of Works. London, H.M. Stationery Office, 1944. 103 pages; 102 plans and photographs. 2s. [£0.1]

This Manual has been compiled with the object of giving technical guidance to local authorities, architects, builders, manufacturers

and others, on the lay-out, planning and construction of permanent houses to be built under the Government's short-term programme covering the first two years after the end of the war in Europe. Local authorities are expected to prepare their own plans in the light of the technical guidance given by this publication.

In July 1944 the Design of Dwellings Sub-Committee of the Central Housing Advisory Committee (appointed by the Ministry of Health) presented its report, and its recommendations have in general been adopted in the Housing Manual. There is detailed discussion of (i) housing and site planning; (ii) the house and its surroundings; (iii) the three-bedroom house; (iv) some special occupants—rural workers, old people, single persons, etc.; (v) flats; (vi) efficiency in building; (vii) new materials and methods; (viii) the heat installation; (ix) services and equipment; there are in addition appendixes on space standards and structural standards. The arrangements for cooking meals usually determine the "way of living" in the working-class house and the plans provided have been evolved from this way of living. These arrangements for cooking will also indicate the fuel or fuels to be used and, to a large extent, the nature and arrangement of the fuel-burning installations and appliances.

One of the major improvements in the lay-out of sites during the past 25 years has been the use of more open forms of development as compared with the close development associated with working-class districts and industrial centres during the nineteenth century. While the plans proposed take into account the value of light and air thus obtained, the establishment of self-contained units or units properly linked to existing communities is envisaged, in order that the tendency to unregulated spreading of sites shall be counteracted. It is urged that provision be made for schools, shopping centres, open spaces, etc., at the outset.

Advice on fuels and appliances has been contributed by the Ministry of Fuel and Power, and the Ministry of Town and Country Planning has advised on site planning and lay-out. The Manual includes many diagrams illustrating different types of plans and drawings of fittings and equipment. A number of excellent photographs of houses already built by local authorities illustrate various arrangements in style.

The Manual is itself an excellent production, set in clear type with carefully drawn plans and excellent half-tone illustrations. A good index is provided.

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A TEXT-BOOK OF PSYCHIATRY

For Students and Practitioners

by D. K. Henderson & R. D. Gillespie. Sixth edition. London, Humphrey Milford, Oxford University Press, 1944. 719 pages. £1 5s. [£1.25]

This book is dedicated to Adolf Meyer, and in general it reflects the biological interpretation of mental disease associated with Meyer and the North American school, while giving due attention to the work of the continental psychiatrists. Dr. Henderson is Professor of Psychiatry in the University of Edinburgh and Dr. Gillespie is Physician for Psychological Medicine at *Guy's* Hospital. This is an excellent publication and is deservedly recognized as the standard British text-book on psychiatry. Its scope is shown by its chapter headings: (i) historical review of the care and treatment of mental illness; (ii) classification; (iii) ætiology; (iv) method of examination; (v) symptomatology; (vi) general psychopathology; (vii) psychoneurotic reaction-types; (viii–ix) affective reaction-types; (x) schizophrenic reaction-types; (xi) paranoia, paraphrenia and paranoid reaction-types; (xii) psychopathic states; (xiii) special methods of physical treatment; (xiv) the organic reaction-types; (xv) epilepsy; (xvi) mental defect; (xvii) psychoses and psychoneuroses in war; (xviii) the psychiatry of childhood; (xix) occupational therapy; (xx) relations of psychiatry and law.

In the new edition the authors have taken account of the increasing incidence of the psychoneurotic forms of reaction; a special chapter on physical methods of treatment records the great advances made in these methods during the past few years, and carefully examines the results obtained by their use; the discussion on epilepsy has been amplified; in the light of their experience the authors have now modified Kraepelin's classification of the schizophrenic states, the paranoid states being removed from the group; the social aspects of psychiatry, to which increasing importance is likely to accrue in the future, receive additional commentary in appropriate places throughout the text. There is a wealth of illustrative case-histories, a short bibliography, and numerous references to the literature appear throughout the book. It would perhaps be an advantage to gather these references together, and to incorporate them into a comprehensive bibliography of the subject, to form an important additional section to this invaluable book.

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